STUDIES OF INDUCED RESPIRATORY POLLENOSIS IN THE DOG

By

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This dissertation is dedicated to Parker A. Small, Jr., who has exhibited the patience of a saint and to Carol, someone who has a very high regard for education.

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(a 1:30 dilution bound 50% of labeled SPE). Symbols as in figure 13.

Resting pulmonary function values were:

	Ra	Vt	Cd	PEFR	RR
A	0.51	0.30	0.62	0.97	8
B	0.43	0.43	0.48	1.06	10

Response of animal G422 to bronchial challenge with 4.75 mg protein nitrogen SPE on day 0 (A), day 14 (B), 2 hours after administration of 65 ml (approximately 550 mg) of a solution of rabbit anti-SPE (a 1:30 dilution bound 50% of labeled SPE), day 28 (C), day 42 (D), and day 56 (E), 2 hours after administration of 50 ml (approximately 475 mg) of a solution of dog anti-SPE (a 1:40 dilution bound 50% of labeled SPE). Symbols as in figure 13.

Resting pulmonary function values were:

	Ra	Vt	Cd	PEFR	RR
A	1.54	0.49	0.15	1.60	13
B	1.16	0.37	0.19	1.48	20
C	1.59	0.57	0.24	1.46	8
D	1.42	0.33	0.14	1.09	18
E	1.64	0.42	0.29	0.73	14

Response of animal YW383 to bronchial challenge with 4.75 mg protein nitrogen SPE on day 0 (A), day 14 (B), 2 hours after administration of 62 ml (approximately 530 mg) of a solution of rabbit anti-SPE (a 1:30 dilution bound 50% of labeled SPE), day 28 (C), day 42 (D), day 56 (E), 2 hours after administration of 46 ml (approximately 430 mg) of a solution of dog anti-SPE (a 1:40 dilution bound 50% of labeled SPE) and day 176 (F). Symbols as in figure 13.

Resting pulmonary function values were:

	Ra	Vt	Cd	PEFR	RR
A	1.03	0.45	0.18	1.96	8
B	0.72	0.60	0.26	2.02	24
C	1.19	0.45	0.40	1.58	8
D	1.07	0.52	0.25	1.72	9
E	1.18	0.67	0.24	1.15	12
F	1.49	0.46	0.22	0.96	12

Response of animal RB396 to bronchial challenge with 4.75 mg protein nitrogen SPE on day 0 (A), day 14 (B), 2 hours after administration of 79 ml (approximately 670 mg) of a solution of rabbit anti-SPE (a 1:30 dilution bound 50% of labeled SPE), day 28 (C), day 42 (D), day 56 (E), 2 hours after administration of 60 ml (approximately 570 mg) of a solution of dog anti-SPE (a 1:40 dilution bound 50% of labeled SPE) and day 176 (F). Symbols as in figure 13.

Resting pulmonary function values were:

	Ra	Vt	Cd	PEFR	RR
A	1.02	0.54	0.46	0.95	16
B	1.78	0.55	0.25	0.97	9
C	1.60	0.68	0.19	1.57	8
D	1.07	0.50	0.33	1.49	20
E	1.34	0.65	0.23	1.53	15
F	1.59	0.75	0.45	1.13	18

Resting pulmonary function values were:

	Ra	Vt	Cd	PEFR	RR
A	0.65	0.54	0.29	1.37	5
B	1.70	0.40	0.18	1.30	26

Abstract of Dissertation Presented to the
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STUDIES OF INDUCED RESPIRATORY POLLENOSIS IN THE DOG

By

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Major Department: Immunology and Medical Microbiology

This investigation resulted in the development of an animal model for the study of respiratory allergic phenomena. A technique for inducing hypersensitivity to prairie sage pollen (Artemisis gnopheles) in the dog was developed. The allergen induced skin reactivity in most dogs and respiratory hypersensitivity in 7 of the 17 animals tested. As skin sensitivity increased bronchial sensitivity tended to increase also. The respiratory hypersensitivity resembled naturally occurring respiratory allergies in both man and dog. The route of allergy induction resembles the natural route and it is felt that this system provides a useful model for the study of respiratory allergies.

This model system was used to investigate several aspects of allergic phenomena. Animals in which respiratory hypersensitivity was induced were used to investigate the role of passive "blocking" antibody in respiratory allergy. It was observed that passive antibody will greatly inhibit the respiratory response to inspired allergen while

completely inhibiting the cutaneous response.

The time required for regeneration of reactivity in skin sites initially reacted with sage pollen extract of anti-IgE was investigated by hypersensitive dogs. It was observed that the time required for regeneration of target organ reactivity was dependent upon the initial degree of sensitivity of the animal.

Initial, partial characterization of this allergen system was performed. The allergen was found to be divisable into four populations by anion exchange chromatography or by molecular seive chromatography. Analytical polyacrylamide gel electrophoresis revealed the allergen to be composed of at least nine components. The allergen system was found to be not greatly different from other pollen allergen systems which have been described.

INTRODUCTION

Respiratory allergies constitute a significant health problem affecting a large portion of the population (1,2). Of the respiratory allergies, asthma is perhaps the most compromising. It is estimated that active asthma afflicts approximately 4% of the American population and another 3% have had it previously (3). Although the annual mortality from asthma is relatively small (about 4000 deaths/year) the disease accounts for 5% of all chronic disabilities.

Asthma places a large burden on our health care system. Statistics for the year 1967 (3) illustrate the extent of this burden. In 1967 there were 1,078,000 days of hospital care for asthmatics at a cost of $83 million and there were 10,181,000 physician visits at a cost of $81 million. The total direct cost of asthma in 1967 was $243 million. In addition to the direct effects of asthma, the disease resulted in 17.5 thousand man years lost from work and house keeping in 1967. The total cost, direct plus indirect, came to a staggering $515 million.

Allergic phenomena have been investigated extensively both in man and in various animal species, and while there have been a number of animal models of immediate hypersensitivity described, ranging from mice to monkeys, no ideal model system has yet been described. The most popular species for the study of allergic phenomena appear to be the mouse, the rat, the guinea pig, the rabbit, non-human primates and the dog.

In many respects mice serve nicely as biologic models. They are

small and relatively inexpensive to obtain and keep. In addition, inbred strains allow for the study of large genetically uniform populations. The immune system of the mouse is perhaps more fully studied and better understood than that of any other animal. Given these facts, it would appear that the mouse would lend itself to the study of allergic disease.

Indeed, there have been a number of studies of immediate hypersensitivity responses carried out in mice and mice have been shown to possess an IgE-like homocytotropic antibody (4,5) as well as the 7S γ_1 skin fixing antibody known for some time (4-9). The discovery of mouse IgE enhances the usefulness of mice in the studies of allergic disease. However, there are several disadvantages in the mouse model system when applied to respiratory allergies. Many of the studies on immediate hypersensitivity in the mouse have involved antigens other than natural allergens administered by parenteral routes (not the natural route of sensitization in respiratory allergy), and the technology does not presently exist to follow parameters of respiratory function following bronchial challenge in the mouse. Even considering these drawbacks, several recent studies have shown the usefulness of the mouse in studying certain facets of respiratory allergic disease. It has recently been shown that mice can be sensitized to natural aeroallergens by the parenteral route (10) and can be sensitized by the respiratory route both to protein antigens (11) and pollen allergens (12). Perhaps the most interesting of these studies is that of Chang and Gottshall (12) who induced systemic sensitivity to ragweed pollen by injecting mice with pertussis vaccine or infecting mice with live pertussis organisms followed by a series of aerosol treatments with ragweed pollen. One

of these aerosol treatments consisted of simply housing mice in an environment naturally contaminated with ragweed pollen. Several other recent studies indicate two areas where mice could be very useful in the study of respiratory allergies. The first of these two areas involves specific inhibition or suppression of the formation of reaginic antibodies to specific allergens. Several recent studies have shown promise in this area (13-19). Secondly, mice seem especially well suited for use in studying the genetics of allergic disease (20,21).

Rats have been used in some laboratories in recent years to study immediate hypersensitivity phenomena. The rat offers many of the same advantages as the mouse as a biomedical model. The main differences are that the rat is larger than the mouse, there are not as many inbred strains of rats as mice and the immune system of the rat is not as thourghly investigated as is that of the mouse.

In recent studies rats have been shown to be able to mount immediate hypersensitive responses to a number of antigens (including natural allergens) and the mediating class of antibody has been shown to resemble IgE (22-24). In addition Von Hout and Johnson (25) induced homocytotropic antibodies in rats by aerosol exposure to bovine serum albumin (BSA) in conjunction with i.p. injection of Bordetella pertussis vaccine. Not only may the rat be sensitized by the respiratory route but the technology now exists to measure parameters of respiratory physiology following antigen challenge in the rat (26-38). These studies have employed intravenous challenge but it should not be too difficult to devise a method of bronchial challenge. To date, the rat has lent itself primarily to the study of three areas of allergic disease. These

are the effect of pharmacologic agents on the allergic response (26), the induction of tolerance or the suppression of the IgE antibody response to specific antigens (29-32) and studies on the genetic control of reagin synthesis (33).

The guinea pig was perhaps the first animal model to be used to study respiratory hypersensitivity, being used for this purpose as early as 1917 (34). In early experiments it was shown that guinea pigs could be sensitized by the inhalation of allergens such as horse dander and pollen (34-36). Ratner (35) showed the induction of asthma-like reactions in guinea pigs by inhalation of dry pollen. More recently Popa, Douglas and Bouhuys (37) have shown positive respiratory responses in guinea pigs sensitized to egg albumin. Other studies have shown that guinea pigs may be sensitized to various protein antigens and nematode parasite antigens (38-41). The mediating antibody class in the guinea pig has been shown to be IgE (40,41). The guinea pig model has been utilized to study several aspects of respiratory hypersensitivity including antigen localization in the respiratory tract (39), the effect of pharmacologic agents on the respiratory response (37,41,42) and specific and non-specific passive desensitization (43-45).

The rabbit, long popular for immunological studies, has been utilized to investigate several aspects of immediate hypersensitivity phenomena. Rabbits have been shown to produce reaginic antibody in response to injection with a number of proteins and hapten-protein conjugates (46-49). This antibody has been shown to belong to the IgE class of immunoglobulin (46-49). In addition, it has been shown that rabbits will produce reaginic antibody in response to nasal instillation of pollen (50). The rabbit model system has been utilized to investigate the ontogeny of the reaginic

response (51) and the suppression of reagin synthesis by passively administered specific antibody (52).

Non-human primates have a relatively close phylogenetic relationship to man and therefore are popular models for the study of many biomedical phenomena, including allergic responses. Non-human primates have been shown to produce reaginic antibodies in response to both naturally occurring and induced parasite infestations (2,3,53-55). In addition, monkeys have been shown to produce reaginic antibodies as a response to injection with hapten-protein conjugates (56) and pollen extracts (57). The reaginic antibody produced in monkeys has been shown to be of the IgE class (57-59), and so closely resembles human IgE that it is antigenically cross-reactive with human IgE (57,59).

Non-human primates have lent themselves to the study of several aspects of respiratory allergic disease. Monkeys actively sensitive to parasite allergens will respond with a positive bronchial response when challenged with aerosols of these antigens (53,55,60) thus providing a model for the study of the respiratory response. Studies have also shown that monkeys may be sensitized for cutaneous, systemic or respiratory responses by passive administration of either monkey or human serum from sensitive individuals (2,55,61,62). This allows for a model system, that in some respects, is more easily obtainable than animals actively sensitized to parasites. In addition, when serum from pollen sensitive humans is utilized to sensitize monkeys, the antigenic system is that normally found in human respiratory allergic disease. These model systems have been utilized to study the effect of pharmacologic agents on the allergic response (55), the changes in arterial oxygen tension as a result of respiratory response (61,63) and the

bronchial cellular exudate following respiratory response (53).

The dog has been utilized extensively to investigate allergic phenomena. Perhaps the most important single factor leading to the popularity of the dog for these types of studies is the fact that naturally occurring allergies in dogs are well documented (58,64-70) and the fact that the dog is the only animal other than man in which atopic disease is known to occur due to aeroallergens (55). Based on cutaneous hypersensitivity testing dogs have been shown to have naturally occurring sensitivities to a wide variety of allergens including pollens, danders, feathers, house dust and insect allergens (71,72). Clinical allergic disease has been reported in dogs caused by food allergens (58,66), parasitic allergens (55,64,73) and pollen allergens (2,55,65,67-70,74-76). The pollen which most commonly is reported to cause allergic disease in dogs is that of ragweed (2,55,65,70,74,75,77).

Naturally occurring pollenosis in the dog has been extensively studied and resembles pollenosis in man (2,65,74,78-80). The serum mediator of the allergic reaction in the dog has been shown to be IgE as it is in man (2,80-84). Clinically pollenosis in the dog usually manifests itself as intensely pruritic dermatitis, conjunctivitis or rhinitis. The animal may have one or any combination of these symptoms. More rarely dogs may have pollen allergics which result in asthma. In addition, dogs which have naturally occurring allergic disease, which does not include asthma, may be induced to have asthmatic symptoms by aerosol challenge with sufficient quantities of the offending allergen, be it pollen (2,55,65) or parasite extract (77).

Naturally occurring allergic disease in the dog offers a model system which has been utilized to investigate various aspects of the

symptom complex. One factor that has facilitated the use of naturally sensitive dogs is the ability to passively transfer sensitivity from naturally sensitive dogs to non-sensitive dogs (2,61,64,74,78). This model systems (naturally sensitive dogs or passively sensitized dogs) has been utilized to investigate the physiology of the allergic response (cutaneous, systemic and respiratory) (2,55,61,64,65,73,74,77, 85), the effect of pharmacologic agents on the allergic response (55,65), changes in arterial oxygen tension as a result of allergic responses (63) and the clinical management of the disease state (68,70).

In addition to naturally allergic dogs, another source of dogs with immediate hypersensitivity exists. A number of studies have been carried out which show that dogs may be induced to produce reaginic antibody by the injection of various antigens including proteins (86, 87), hapten-protein conjugates (84,86,88) and pollen allergens (89-92). It has also been shown that atopic dogs can be sensitized to a hapten by aerosol exposure to a hapten-pollen conjugate (93). The reaginic antibody induced in these studies resembles that occurring in natural allergies, has been shown to be IgE (84,86,87,89,91,93) and can be passively transferred to normal dogs (84,86,87,89,93). Challenge studies in the induced hypersensitive dog have had mixed results. Systemic (i.v.) and respiratory challenge resulted in negative responses in the studies of Arkins et al. (89) and Sunthonpalin et al. (91). Dhaliwal et al. obtained positive systemic responses in dogs with induced sensitivity to ragweed pollen following i.v. challenge with ragweed pollen extract (92). Finally, Kepron et al. (88) produced positive respiratory responses following bronchoprovocation in dogs with induced sensitivity to 2,4-dinitrobenzene. This model system of induced hypersensitivity

has been utilized to study the ontogeny of the allergic immune response (88).

The animal is not the only important part of the model system. It was also necessary to give careful consideration to the allergen to be used in the system. In studying reaginic responses in various animal species a number of antigens have been utilized including proteins such as BSA and haptens such as DNP. A limited number of studies have employed naturally occurring allergens. It was felt that in developing a model system, which would simulate naturally occurring allergies, a natural allergen should be employed. The allergen chosen for these studies was the pollen of prairie sage (sage pollen). This pollen was chosen because it is strongly antigenic (94) and natural sensitivity to it in the dog has been demonstrated (70).

These studies report the development of a model system in which the induction of skin and bronchial sensitivity to pollen was accomplished. This model system was utilized to study the role of "blocking" antibody in respiratory allergies and it was found that passively administered antibody suppressed the respiratory response to allergen challenge. The allergen system used was partially characterized and was found to possibly be somewhat more restricted in its component makeup than other pollen systems which have been studied.

MATERIALS AND METHODS

Animals

Neonatal (10 days to 6 weeks old at the initiation of study) and adult mongrel dogs were purchased from, and housed by, the Health Center Animal Research Department of the University of Florida, Gainesville, Florida. All of the adult dogs utilized in this study were skin tested with prairie sage pollen extract before being placed in the study. Only skin test negative animals were used. Approximately 70% of the animals tested were skin test negative.

Allergens

The pollen of prairie sage (Artemisia gnopheles), referred to henceforth as sage pollen, was chosen for these studies because it is strongly antigenic (94) and is responsible for naturally occurring sensitivity in dogs (70). Pollen was purchased initially from International Biologics Inc., Bethany, Oklahoma. The final respiratory challenge was carried out using pollen from Greer Laboratories, Inc., Lenoir, North Carolina. The latter product appeared more homogeneous than the former when examined microscopically. Pollen suspension used in sensitizing treatments was made immediately prior to use. This was done to avoid extraction of water soluble components from the pollen.

For the majority of experiments, pollen extract was produced by extracting pollen with phosphate buffered saline (PBS). In initial studies, pollen was extracted by the method of Coca (95) as described by Phillips (96). Before use in bronchial challenge this extract was

dialyzed extensively against PBS to remove phenol.

Sensitizing Regimen

The animals were sensitized as shown in Table I and II with sage pollen in the following manner. Both adult and neonatal animals were divided as follows: 5 adults and 3 neonates received no treatment: 5 adults and 5 neonates received 1.3×10^{10} Bordetella pertussis organisms/ treatment subcutaneously (SC) twice weekly for 3 weeks, 3 adults and 4 neonates received pollen suspended in normal saline intranasaly (0.1 to 0.4 mg pollen/treatment/animal) and B. pertussis (1.3×10^{10} organisms/ treatment/animal) SC twice weekly for 3 weeks; and 15 adults and 4 neonates received pollen suspended in normal saline intranasaly (0.1 to 0.4 mg pollen/treatment/animal_ twice weekly for 3 weeks.

Pulmonary Function Evaluation

Resistance of the respiratory airways, dynamic compliance of the lungs, tidal volume and peak expiratory flow rates were the parameters used to evaluate pulmonary functions.

The dogs were anesthesized with sodium pentobarbital (Burns-Biotec Laboratories, Inc., Oakland, CA), intubated and placed in ventral recumbancy after which an esophagel balloon catheter was positioned at a point where the pressure was the most negative. The following parameters were monitored on a polygraph recorder (Brush Accuchart, Gould, Inc., Cleveland, OH): 1) air flow at the end of the tracheal tube with a Fleisch pneumotachograph and a differential pressure transducer (Stalham PM 285TC); 2) tidal volume by integration of the respiratory flow rates; and 3) trans-airway pressure (difference between esophagel and trachcal tube pressure) with a differential pressure transducer.

Respiratory resistance was calculated according to the method of

Amdur and Mead (97) in which the trans-airway pressure differences (P) at isovolume points (approximately mid volume) during inspiration and expiration is divided by the sum of the air flow at these two points (figure 1):

$$\text{Resistance} = \frac{P_3 - P_4 \text{ cm } H_2O}{\dot{V}_1 + \dot{V}_2 \text{ L/sec}}$$

Dynamic compliance was calculated by dividing the difference in volume between the 2 points where air flow was zero by the difference in pressure at these points (figure 1):

$$\text{Compliance} = \frac{V_2 - V_1 \text{ L}}{P_2 - P_1 \text{ cm } H_2O}$$

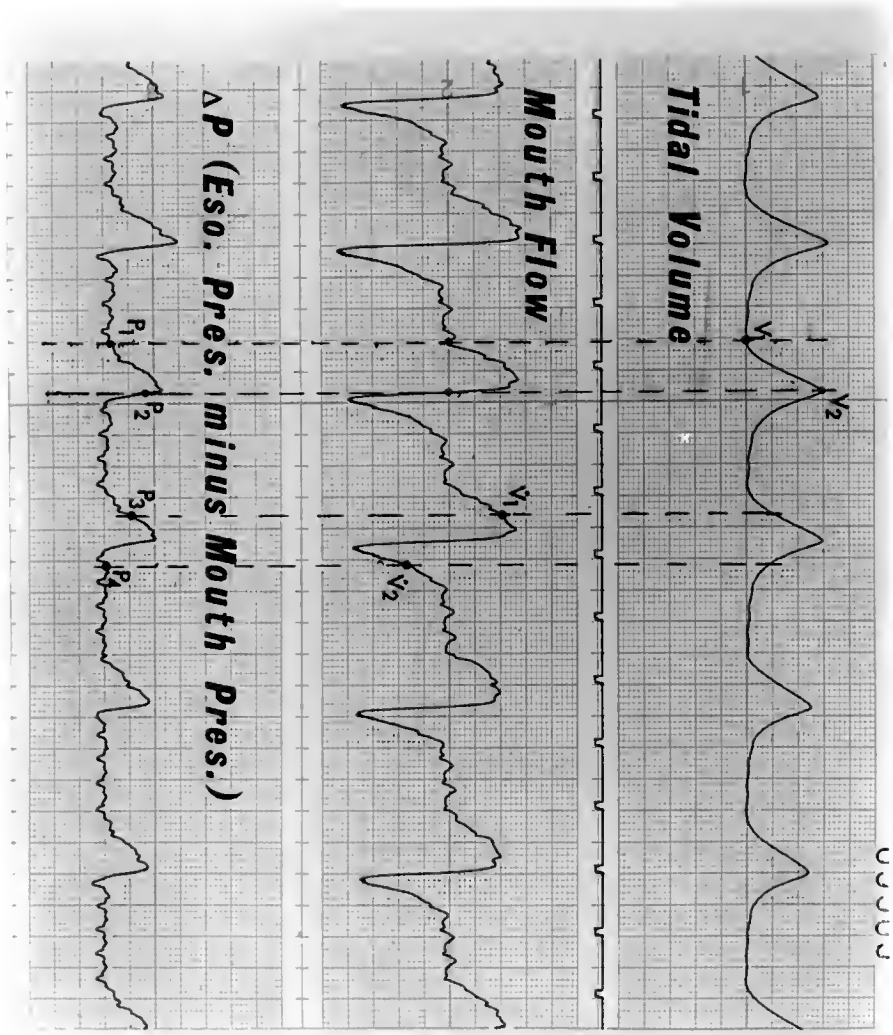
Respiratory function was evaluated prior to bronchial challenge and at 5 to 10 minute intervals, for up to approximately 30 minutes following challenge. Values for respiratory resistance, dynamic compliance, tidal volume and peak expiratory flow rates were determined by averaging calculations made from 15 consecutive respiratory cycles during each evaluation period. The animals lungs were hyperinflated prior to each evaluation period in order to prevent atelectasis and maintain a baseline.

Respiratory resistance was the parameter utilized to determine whether or not an animal was sensitive. An increase in respiratory resistance greater than 35% was arbitrarily taken to indicate a positive bronchial response. Respiratory resistance never increased more than 8% when normal dogs were challenged with SPE.

Methods of Challenge

One week after the last sensitizing treatment the animals were

Figure 1 Typical polygraph tracing from which physiologic data was obtained. Of the 5 parameters monitored, 3, tidal volume, peak expiratory flow rate, and respiratory rate may be read directly from the chart.


Tidal Volume
V1
V2
Mouth Flow
V̇1
V̇2
ΔP (Eso. Pres. minus Mouth Pres.)
P1
P2
P3
P4

tested for skin reactivity with varying dilutions of SPE. The animals were bronchially challenged within 2 weeks after the last sensitizing treatment.

Skin tests were performed by injection of 0.1 ml of varying dilutions (950, 95, 9.5 and 0.95 µg protein nitrogen/ml) of SPE intradermally followed by 1.5 ml of a 1% solution of Evans blue i.v. Skin sites were observed for blueing at 15 and 30 minutes post-injection. Any skin sites showing a blueing reaction with a diameter greater than 5mm was scored as a positive reaction.

Bronchial challenge was accomplished by delivering 1 ml of challenge material over a 5 minute period through a Bird micro-nebulizer connected to the endotracheal tube and driven by a Bird Mark VIII respirator actuated by a pressurized gas mixture of 5% CO_2, 20% O_2 and 75% N_2. Peak inspiratory pressure during nebulization was 25cm H_2O and the rate was approximately 30 breaths/minute. The respirator and nebulizer were removed before recording of physiologic parameters. The animals were initially challenged with PBS as a control, followed by challenge with varying concentrations of histamine (Histamine Hcl, Fisher Scientific Co., Pittsburgh, PA) or approximately 4.75 mg protein nitrogen of SPE.

Sample Collections

Blood samples for serum harvest were collected by femoral venipuncture immediately prior to skin testing. Arterial blood samples, for blood gas determinations, were collected anaerobically via an intracath inserted percutaneously into the femoral artery. Arterial blood PO_2, and PCO_2 and pH were determined with a blood gas analyzer (model 113 Blood Gas Analyzer, Instrumentation Laboratory, Inc., Lexington, MA).

Nasal wash samples were collected by washing the nasal passage with approximately 30 ml of normal saline. These samples were concentrated 10X by vacuum dialysis.

Both serum and nasal wash samples were stored at -20°C until used.

Prausnitz-Kustner Reactions

Prausnitz-Kustner reactions (P.K. reactions) were performed in the skin of normal dogs using undiluted serum and concentrated (10X) nasal wash fluid. These reactions were run in duplicate in 2 normal dogs (i.e., 4 skin reactions/sample). The hair was clipped from the ventrolateral skin and 0.1 ml of the sample to be tested was injected intradermally into skin sites. Forty-eight hours later (78) these skin sites were challenged with 0.1 ml of a solution of SPE containing 95 µg protein nitrogen/ml followed by 1.5 ml of a 1% solution of Evans blue i.v. Challenged sites were read at 15 and 30 minutes post injection. Sites showing blueing at a diameter greater than 5mm were scored as positive.

Antisera

Equine antiserum to human epsilon chain (anti-IgE) was purchased from Kallestad Laboratories, Inc., Chaska, MN. This antisera cross reacts with canine IgE as shown by producing skin reactions in normal dogs at dilutions as high as 1:2048 and as reported by Halliwell, Swartzman and Rockey (81). Antisera to sage pollen extract was produced both in rabbits and dogs. Rabbit antisera were induced by injecting rabbits in multiple subcutaneous sites with approximately 1 mg protein nitrogen of SPE emulsified in complete Freund's adjuvant. These animals were boosted at monthly intervals with the same antigen preparation. Canine antisera were produced by injecting adult mongrel dogs in multiple subcutaneous sites with approximately 2 mg protein nitrogen of SPE

emulsified in incomplete Freund's adjuvant. These animals were boosted with the same antigen at approximately 14 day intervals.

Determination of Sage Pollen Extract Binding Activity

The antibody activity in the rabbit and dog anti-SPE was determined by the technique of Lidd and Farr (98) using ^{125}I-labeled SPE. ^{125}I-SPE was obtained by trace labeling SPE by the chloramine T method as described by McConahey and Dixon (99) with carrier-free ^{125}I (New England Nuclear, Boston, MA).

Quantitation of Rabbit Immunoglobulin

Rabbit IgG was measured using radial-immunodiffusion as described by Mancini, Carbonora and Heremans (100) utilizing goat anti-rabbit IgG purchased from Microbiological Associates, Bethesda, MD.

Fractionation of SPE

SPE was fractionated by anion exchange chromatography using DEAE (diethylamionethyl) cellulose (Whatman DE 32, H. Reeve Angel Inc., Clifton, NJ) columns and by molecular seive chromatography utilizing Sephedex-G25 (Pharmacia Fine Chemicals Inc., Piscataway, NJ) columns. DEAE-cellulose chromatography was performed utilizing columns equilibrated with 0.015 molar Tris, pH 8.2. These columns were eluted with a linear sodium chloride gradient. Molecular seive chromatography was performed in downward flow columns equilibrated with 0.15 molar sodium chlorida, 0.015 molar Tris, pH 7.4.

Electrophoretic Analysis of SPE

Analytical disc electrophoresis was performed on whole SPE and DEAE-cellulose chromatographic fractions of SPE. Electrophoresis was carried out on 15% polyacrylamide gels in the presence of 0.1% Triton X-100 (101).

Determination of Protein Nitrogen Content of SPE

The protein of SPE was determined by the Nessler technique using commercial Nessler's reagent (Fisher Scientific Co., Pittsburgh, Pa).

RESULTS

Response to Bronchial Challenge with Histamine

In order to gain insight into the bronchial sensitivity of dogs to histamine, to determine the approximate changes in respiratory functions required to produce changes in arterial blood gases and to evaluate the bronchial challenge monitoring system, normal dogs were bronchially challenged with varying concentrations of histamine. Figures 2 and 3 depict the respiratory response of 2 normal dogs to bronchial challenge with 1 mg histamine. Both animals responded to this challenge with increases in respiratory resistance (100% and 150% respectively) and decreases in dynamic compliance. These changes were accompanied by decreases in arterial PO_2 and increases in arterial PCO_2.

Figures 4 and 5 illustrate the responses of 2 normal dogs to bronchial challenge with 50 mg histamine. This challenge resulted in severe changes in all respiratory functions measured as well as a pronounced decrease in arterial PO_2 and increase in PCO_2. While the changes in respiratory function were pronounced they were, with the exception of dynamic compliance, only about 15 minutes in duration.

In addition to a single bronchial challenge with histamine, 3 normal dogs were challenged with multiple challenges of histamine (figures 6-8). When these multiple challenges were performed the respiratory response was prolonged and pronounced.

Figure 2 Response of a normal dog to bronchial challenge with 1 mg histamine. A represents changes in parameters of respiratory function. ● = respiratory resistance; ○ = tidal volume; △ = dynamic compliance; ▲ = peak expiratory flow rate; ■ = respiratory rate. B represents changes in arterial blood gases. ● = pCO_2 and ▲ = pO_2.

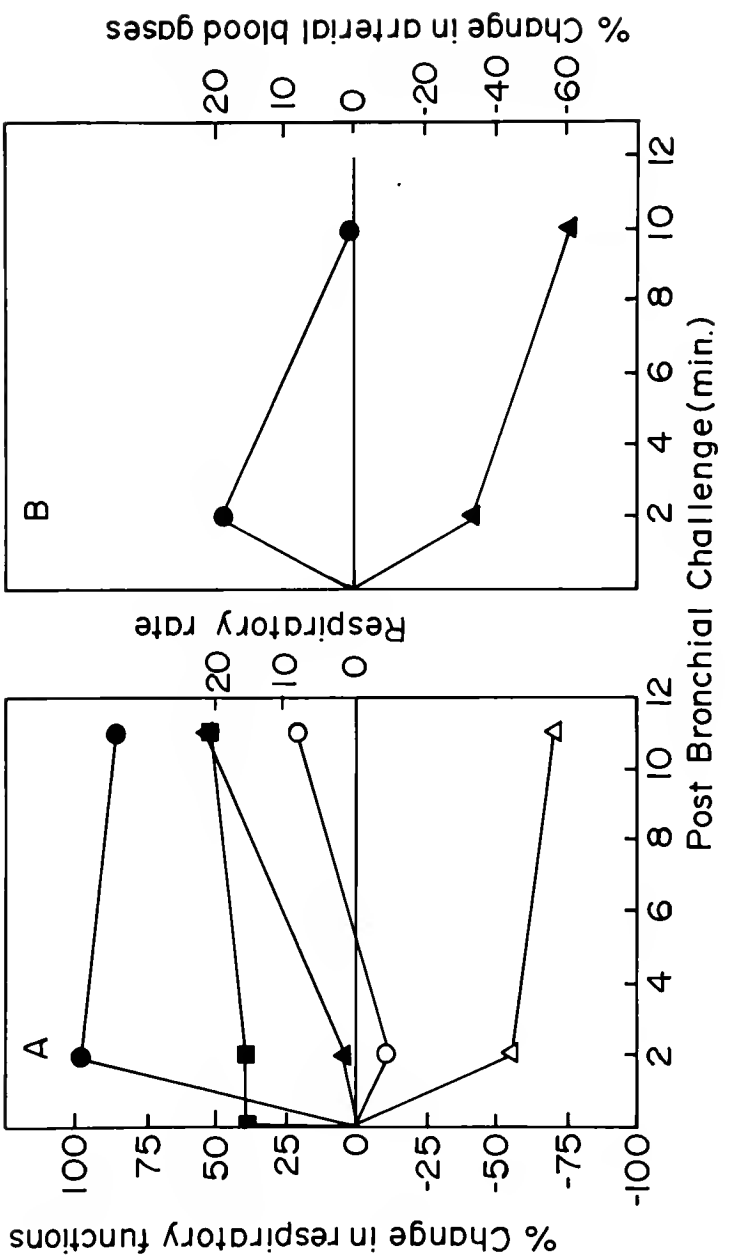

% Change in respiratory functions
100
75
50
25
0
-25
-50
-75
-100
A
2 4 6 8 10 12
Post Bronchial Challenge(min.)
Respiratory rate
20
10
0
B
% Change in arterial blood gases
20
10
0
-20
-40
-60
2 4 6 8 10 12

Figure 3 Response of a normal dog to bronchial challenge with 1 mg histamine. A represents changes in parameters of respiratory function. ● = respiratory resistance; ○ = tidal volume; △ = dynamic compliance; ▲ = peak expiratory flow rate; ■ = respiratory rate. B represents changes in arterial blood gases. ● = pCO_2 and ▲ = pO_2.

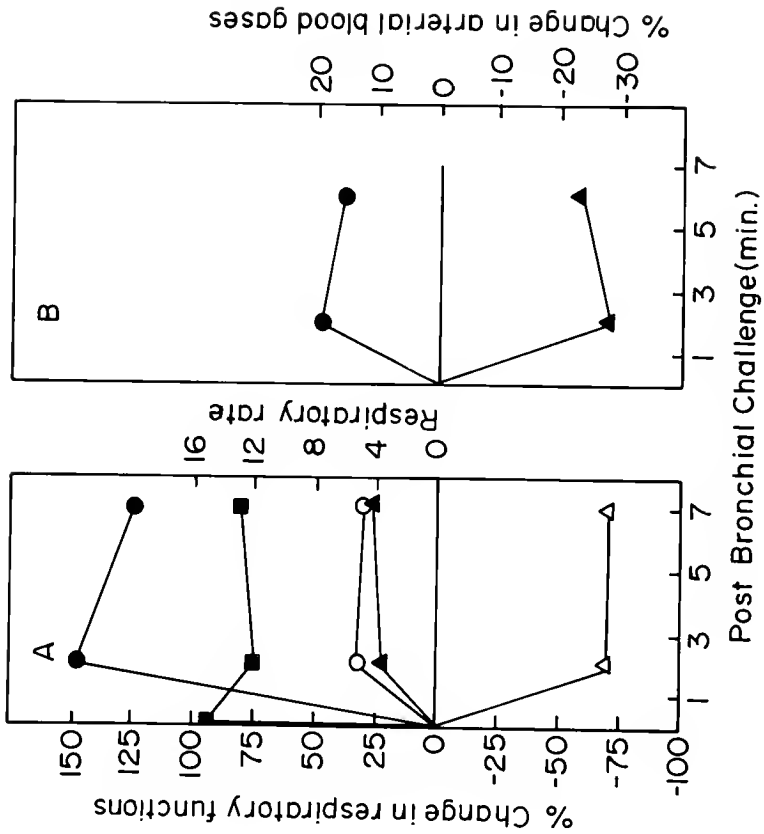

% Change in respiratory functions
150
125
100
75
50
25
0
-25
-50
-75
-100
A
Respiratory rate
16
12
8
4
0
B
% Change in arterial blood gases
20
10
0
-10
-20
-30
1
3
5
7
Post Bronchial Challenge(min.)

Figure 4 Response of a normal dog to bronchial challenge with 50 mg histamine. A represents changes in parameters of respiratory function. ● = respiratory resistance; ○ = tidal volume; △ = dynamic compliance; ▲ = peak expiratory flow rate; ■ = respiratory rate. B represents changes in arterial blood gases. ● = pCO_2 and ▲ = pO_2.

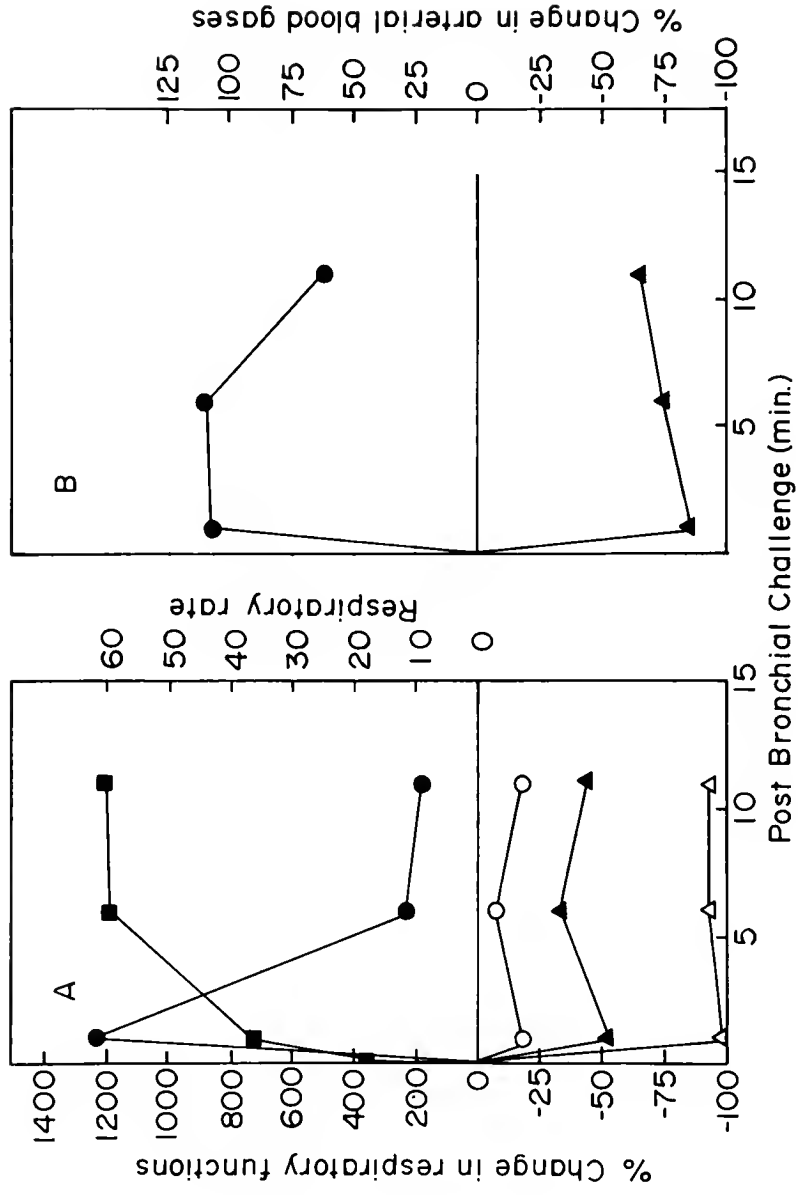

% Change in respiratory functions
% Change in arterial blood gases
Respiratory rate
Post Bronchial Challenge (min.)
A
B

Figure 5 Response of a normal dog to bronchial challenge with 50 mg histamine. A represents changes in parameters of respiratory function. ● = respiratory resistance; ○ = tidal volume; △ = dynamic compliance; ▲ = peak expiratory flow rate; ■ = respiratory rate. B represents changes in arterial blood gases. ● = pCO_2 and ▲ = pO_2.

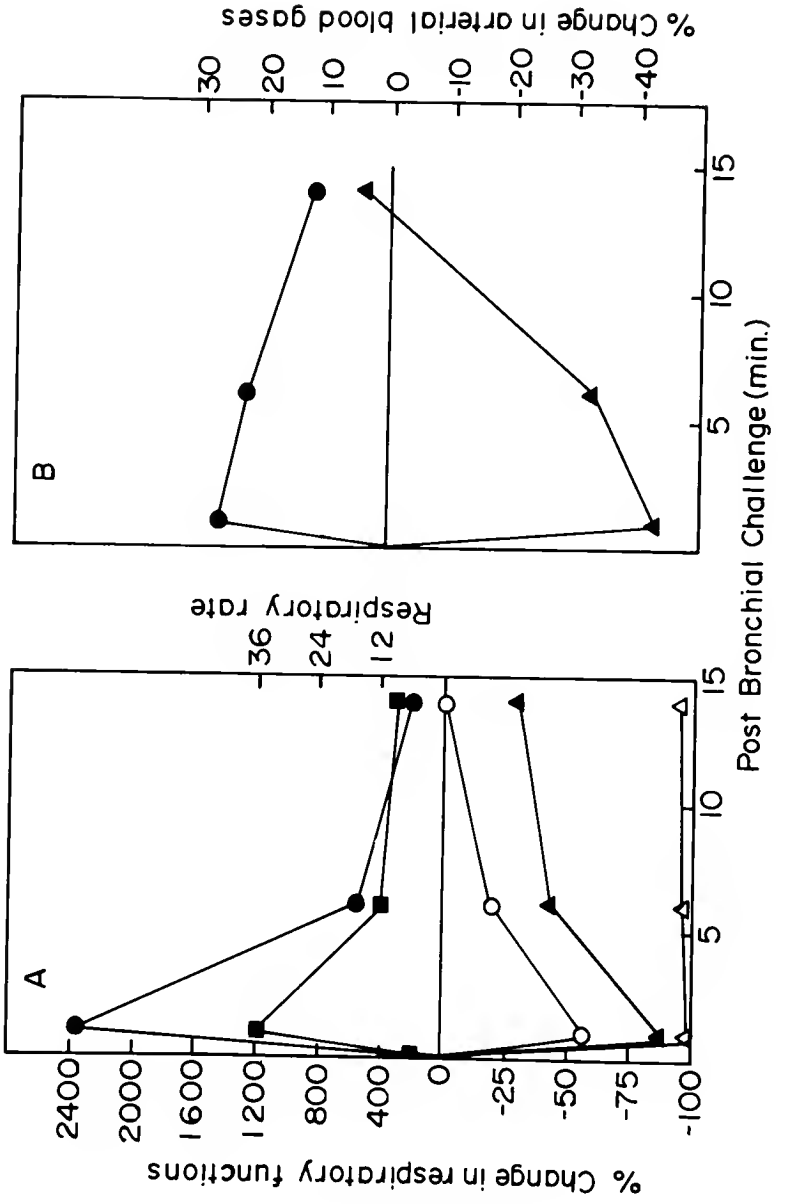

% Change in respiratory functions
2400
2000
1600
1200
800
400
0
-25
-50
-75
-100
A
Respiratory rate
36
24
12
5
10
15
Post Bronchial Challenge (min.)
B
% Change in arterial blood gases
30
20
10
0
-10
-20
-30
-40
5
10
15

Figure 6 Response of a normal dog to multiple bronchial challenge with histamine. The initial challenge was performed with 1 mg histamine and the subsequent challenge with 3 mg histamine. A represents changes in parameters of respiratory function. ● = respiratory resistance; ○ = tidal volume; △ = dynamic compliance; ▲ = peak expiratory flow rate; ■ = respiratory rate. B represents changes in arterial blood gases. ● = pCO_2 and ▲ = pO_2.

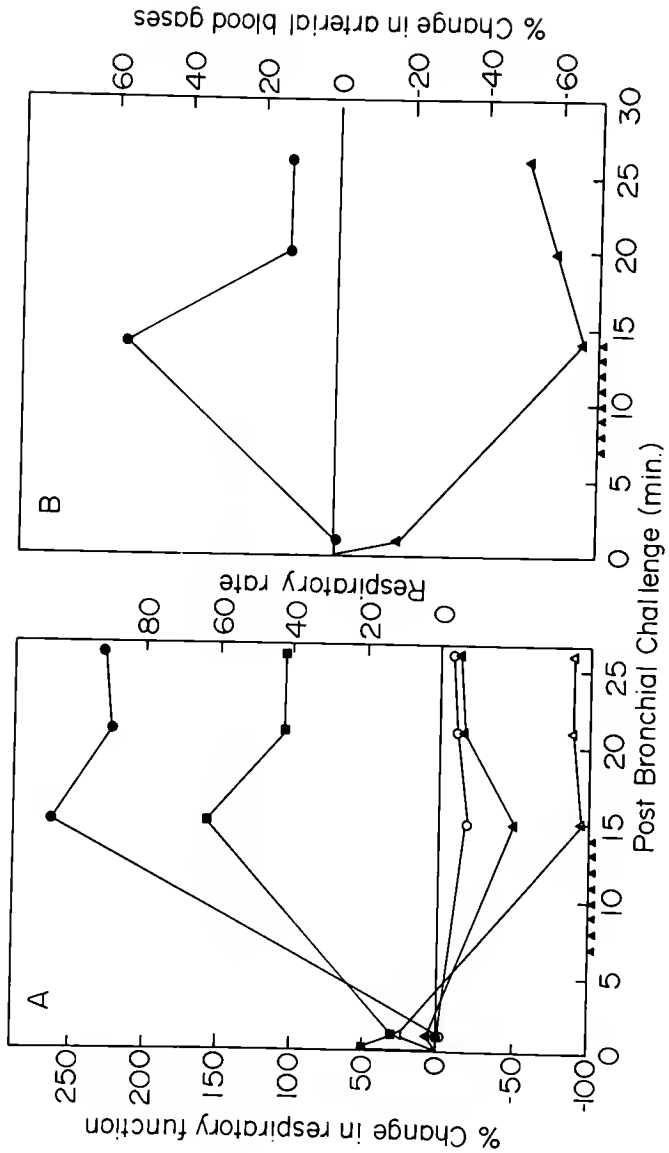

% Change in respiratory function
250
200
150
100
50
0
-50
-100
A
0
5
10
15
20
25
Post Bronchial Challenge (min.)
Respiratory rate
80
60
40
20
0
B
% Change in arterial blood gases
60
40
20
0
-20
-40
-60
0
5
10
15
20
25
30

Figure 7 Response of a normal dog to multiple bronchial challenge with histamine. The initial challenge was performed with 10 mg histamine and the subsequent challenge with 15 mg histamine. A represents changes in parameters of respiratory function. ● = respiratory resistance; ○ = tidal volume; △ = dynamic compliance; ▲ = peak expiratory flow rate; ■ = respiratory rate. B represents changes in arterial blood gases. ● = pCO_2 and ▲ = pO_2.

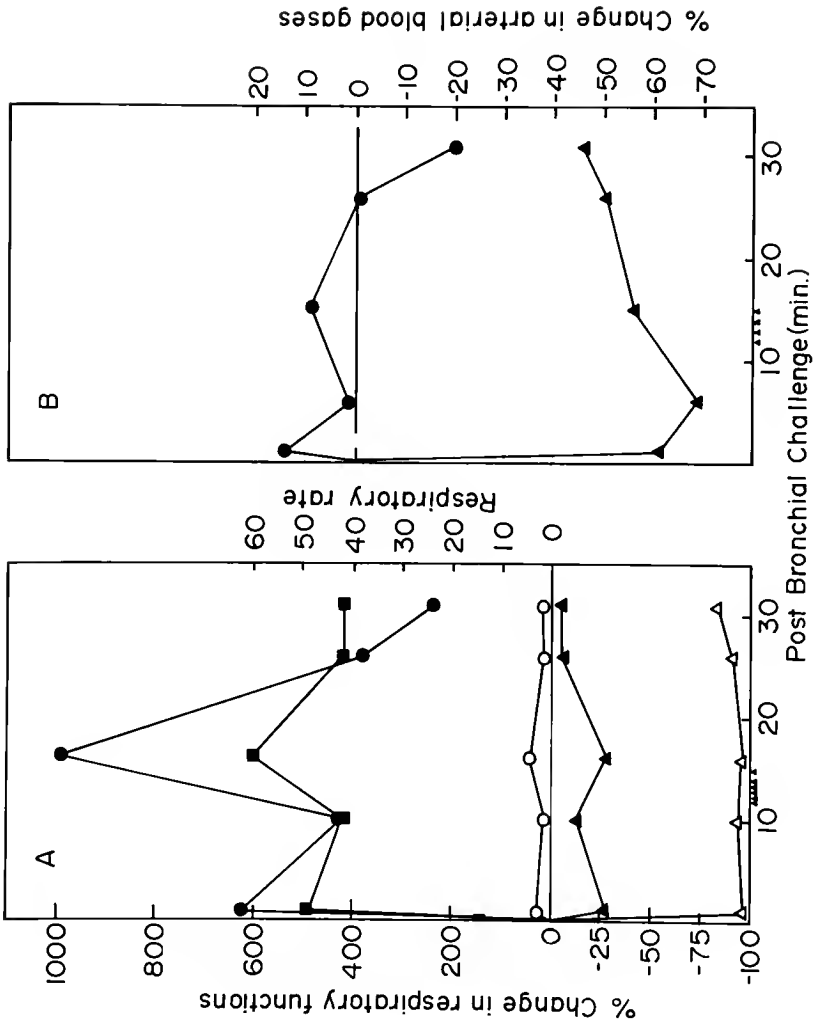
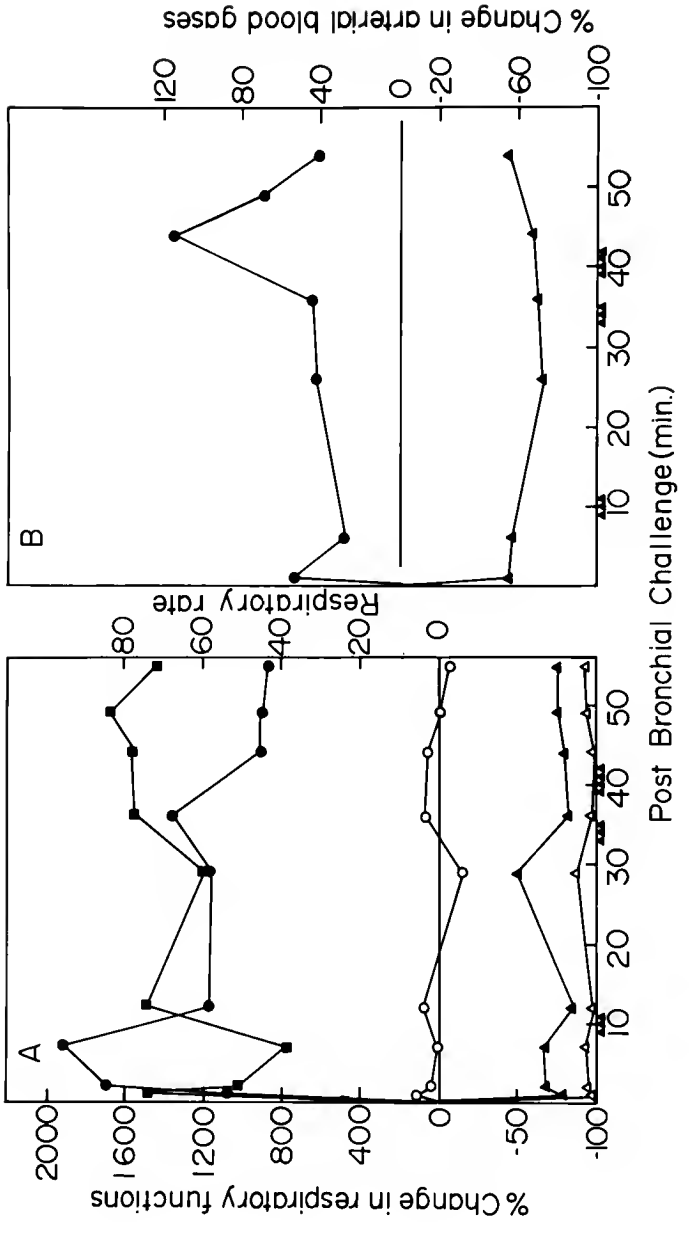

A
B
% Change in respiratory functions
Respiratory rate
% Change in arterial blood gases
Post Bronchial Challenge(min.)
1000
800
600
400
200
0
-25
-50
-75
-100
60
50
40
30
20
10
0
20
10
0
-10
-20
-30
-40
-50
-60
-70
10
20
30

Figure 8 Response of a normal dog to multiple bronchial challenge with histamine. The initial challenge was performed with 50 mg histamine and the subsequent challenges with 100, 50 and 20 mg histamine. A represents changes in parameters of respiratory functions. ● = respiratory resistance; ○ = tidal volume; △ = dynamic compliance; ▲ = peak expiratory flow rate; ■ = respiratory rate. B represents changes in arterial blood gases. ● = pCO_2 and ▲ = pO_2.


% Change in respiratory functions
2000
1600
1200
800
400
0
-50
-100
A
Respiratory rate
80
60
40
20
0
B
% Change in arterial blood gases
120
80
40
0
-20
-60
-100
10
20
30
40
50
Post Bronchial Challenge (min.)

Induction of Sensitivity to Sage Pollen

The initial goal of these studies was to produce pollen hypersensitivity in dogs by a natural route.

Preliminary uncontrolled studies showed that 5 out of 8 neonates and 19 out of 21 adult dogs converted from negative to positive skin tests following intranasal pollen instillation twice weekly for 3 weeks. An additional group of animals received *Bordetella pertussis* at the same time as the intranasal pollen and 14 out of 25 neonates and 16 out of 19 adult dogs converted from negative to positive skin tests. These results were sufficiently encouraging to be followed by controlled experiments.

Table I shows the results obtained in neonatal animals. All 8 of the animals given pollen became positive to skin test, while none of the control animals became positive. All but 1 of skin test positive animals had PK positive serum. This animal (367) was only weakly positive to skin test. Nasal wash PK's were uniformly negative in contrast to the observation of Patterson, *et al*. (77) in dogs sensitive to ascaris but in agreement with their observations in ragweed sensitive dogs (77).

Table II shows results obtained with adult dogs. As can be seen, the majority of animals that underwent sensitizing treatment became sensitive to SPE when measured by skin reactivity or serum PK positivity. Again, all concentrated nasal wash samples were negative in transferring skin reactivity. In addition to inducing skin and serum PK positivity the sensitizing treatments resulted in bronchial sensitivity in 7 of the 13 animals positive by skin and PK tests. This bronchial sensitivity ranged from a minimal sensitivity to a fairly high degree of sensitivity. Figures 9-11 illustrate dogs with varying degrees of bronchial sensitivity.

TABLE I

RESULTS OF SKIN TESTS AND PK REACTIONS OBTAINED FOR NEONATAL DOGS SENSITIZED TO PRAIRIE SAGE POLLEN

Animal Number	Treatment	Skin Test	PK Serum	PK Nasal Wash
363	None	neg.[a]	neg.[b]	neg.
365		neg.	neg.	neg.
366		neg.	neg.	neg.
318	Bordetella pertussis Sub Q	neg.	neg.	neg.
321		neg.	neg.	neg.
322		neg.	neg.	neg.
323		neg.	neg.	neg.
326		neg.	neg.	neg.
317	Pollen Suspension Intranasaly plus B. pertussis Sub Q	pos.	pos.	neg.
320		pos.	pos.	neg.
324		pos.	pos.	neg.
327		pos.	pos.	neg.
364	Pollen Suspension Intranasaly	pos.	pos.	neg.
367		pos.	neg.	neg.
368		pos.	pos.	neg.
369		pos.	pos.	neg.

a.) Any challenged site showing less than 5 mm diameter blueing at 15 and 30 minutes post challenge was scored as negative, sites showing blueing 5 mm or larger in diameter were scored as positive.

b.) Any PK site showing less than 5mm diameter blueing at 15 and 30 minutes post challenge was scored as negative, sites showing blueing 5 mm or larger in diameter were scored as positive.

TABLE II

RESULTS OF SKIN TESTS, PK REACTIONS, AND BRONCHIAL CHALLENGE OBTAINED IN ADULT DOGS SENSITIZED TO PRAIRIE SAGE POLLEN

Animal Number	Skin Test[a]	Treatment	Skin Test[b]	PK Serum	PK N.W.[c]	Bronchial Challenge[d]
YW3118	neg.[d]	None	neg.	neg.[e]	neg.	neg.
YW3103	neg.		neg.	neg.	neg.	neg.
R32	neg.		neg.[f]	neg.	neg.	neg.
YW399	neg.		95µg	neg.	neg.	neg.
YW368			neg.	neg.	neg.	neg.
YW3128	neg.	Bordetella pertussis Sub Q	9.5µg	neg.	neg.	neg.
YW393	neg.		neg.	neg.	neg.	neg.
YW381	neg.		neg.	neg.	neg.	neg.
RB341	neg.		neg.	neg.	neg.	neg.
YW3119	neg.		neg.	neg.	neg.	neg.
GW316	neg.	Pollen Suspension Intranasaly plus B. pertussis Sub Q	9.5µg	pos.	neg.	neg.
YW325	neg.		9.5µg	pos.	neg.	54
RB311	neg.		neg.	neg.	neg.	neg.
YW328	neg.	Pollen Suspension Intranasaly	0.95µg	pos.	neg.	433
YW3104	neg.		0.95µg	neg.	neg.	neg.
GW311	neg.		95µg	neg.	neg.	neg.
YW383	neg.		0.95µg	pos.	neg.	108
G437	neg.		0.95µg	pos.	N.D.[h]	47
G433	neg.		0.95µg	pos.	N.D.	133
G428	neg.		neg.	neg.	N.D.	N.D.
G422	neg.		0.95µg	pos.	N.D.	52
G427	neg.		0.95µg	pos.	N.D.	neg.
RB396	0.095µg[i]		0.095µg	pos.	N.D.	94
G429	neg.		0.95µg	pos.	N.D.	neg.
RB2115	neg.		95µg	neg.	N.D.	neg.
BW326	9.5µg[i]		0.95µg	pos.	N.D.	neg.
G431	neg.		0.95µg	pos.	N.D.	neg.
G424	neg.		0.95µg	pos.	N.D.	neg.

a. Skin test results prior to animal being placed on study.
b. Skin test results post sensitizing treatment.
c. Results of PK reactions with nasal wash samples.
d. Percent increase in respiratory resistance following bronchial challenge.
e. Any challenged site showing less than 5 mm diameter blueing at 15 and 30 minutes post challenge wes scored as negative.
f. Any PK site showing less than 5 mm diameter blueing at 15 and 30 minutes post challenge was scored as negative, sites showing blueing 5 mm or larger in diameter were scored as positive.
g. Lowest concentration in ug protein nitrogen/skin test of SPE giving positive skin test.
h. N.D. indicates not done.
i. Positive skin tests in this column were obtained in animals which were sensitized as neonates and included here in an effort to obtain bronchially positive animals.

Figure 9 Response of 2 minimally responsive dogs to bronchial challenge with 4.75 mg protein nitrogen SPE. ● = respiratory resistance; ○ = tidal volume; △ = dynamic compliance; ▲ = peak expiratory flow rate; and ■ = respiratory rate.

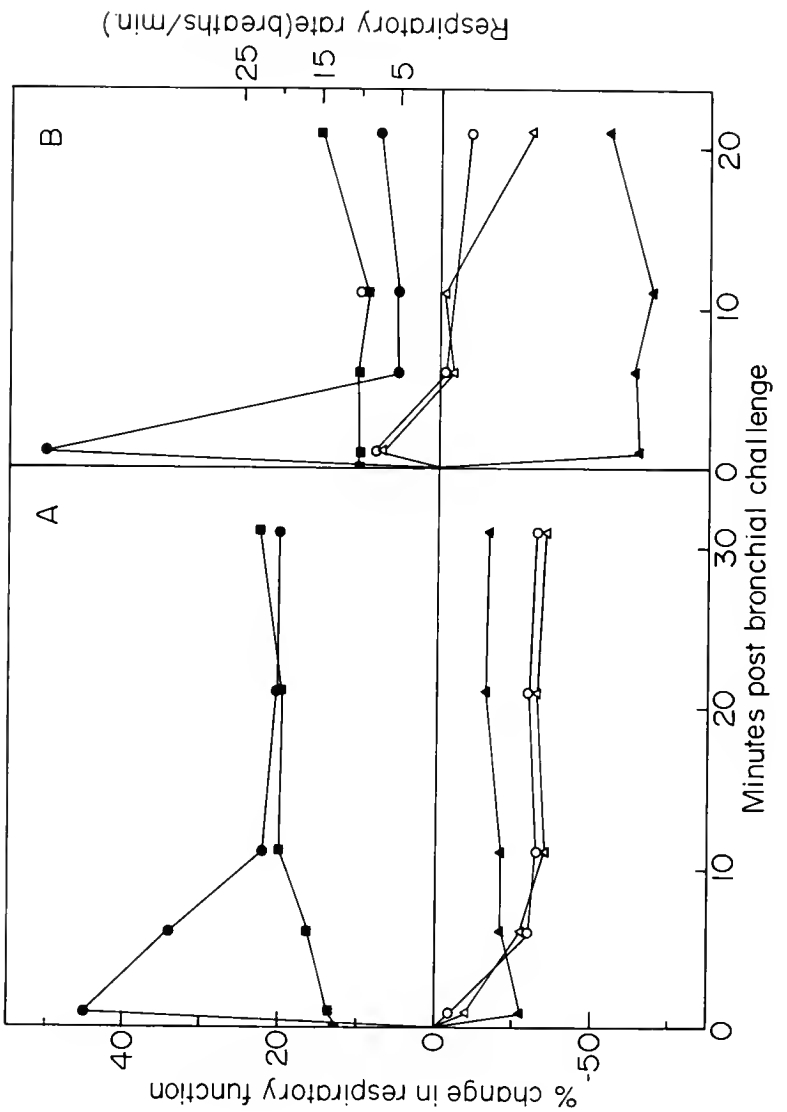

Respiratory rate(breaths/min.)
25
15
5
B
A
% change in respiratory function
40
20
0
-50
0
10
20
30
0
10
20
Minutes post bronchial challenge

Figure 10 Response of 2 medially responsive dogs to bronchial challenge with 4.75 mg protein nitrogen SPE. ● = respiratory resistance; ○ = tidal volume; △ = dynamic compliance; ▲ = peak expiratory flow rate; and ■ = respiratory rate.

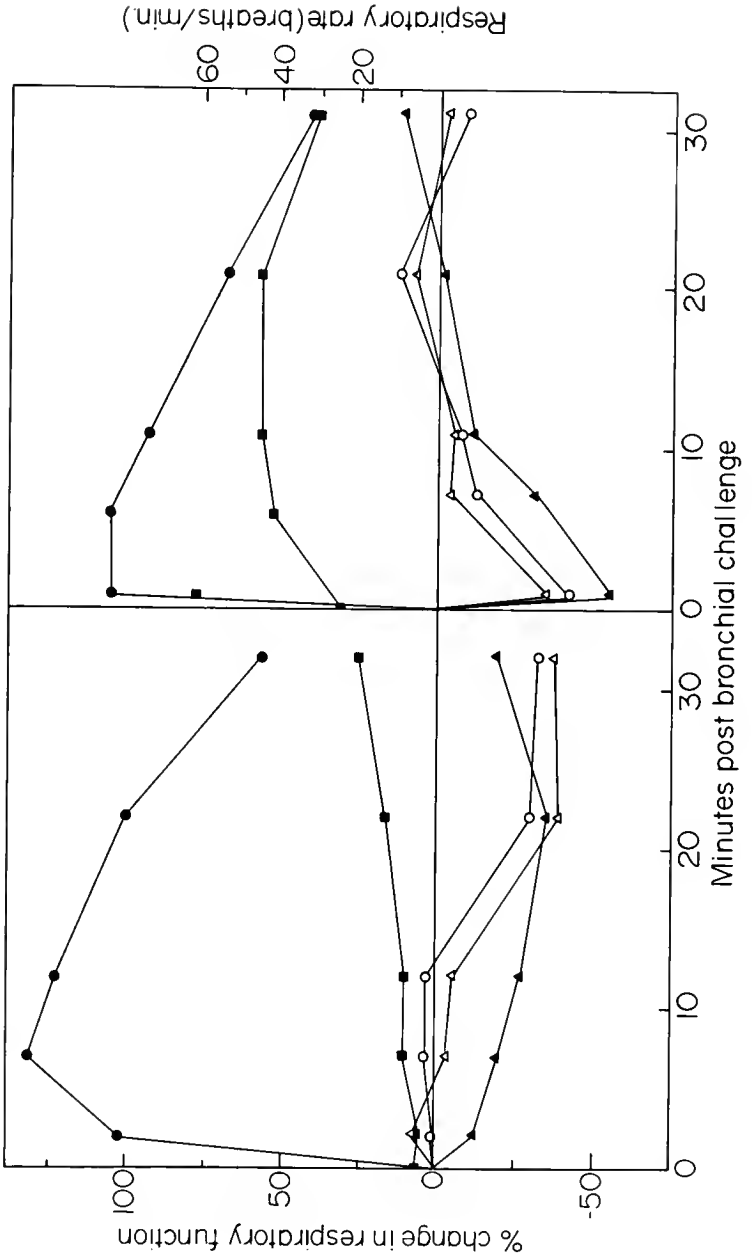
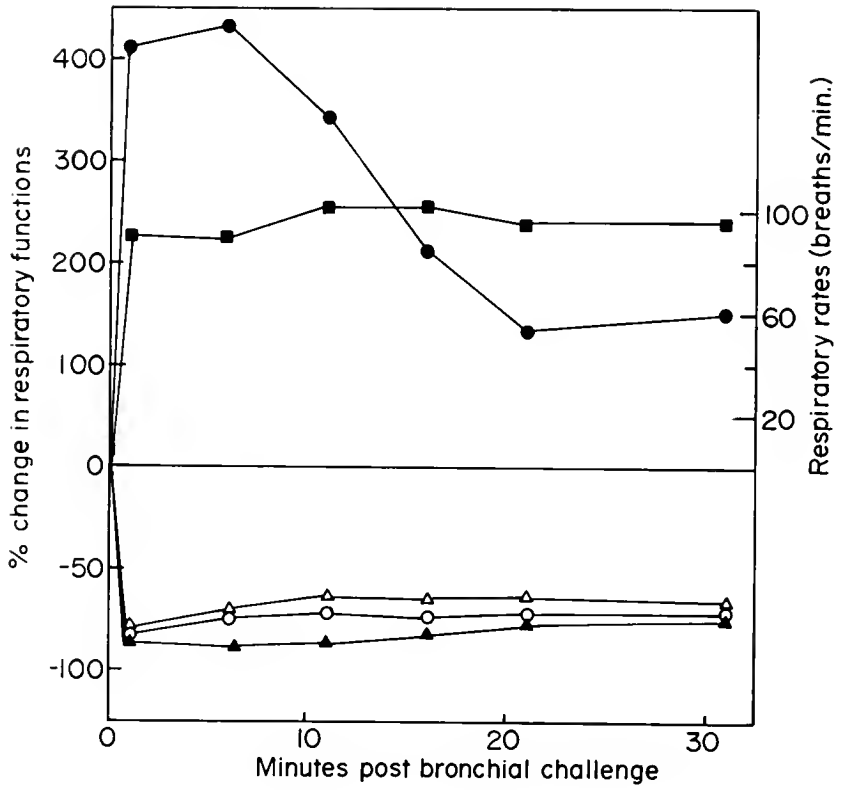

% change in respiratory function
100
50
0
-50
0
10
20
30
0
10
20
30
Minutes post bronchial challenge
Respiratory rate (breaths/min.)
60
40
20

Figure 11 Response of a highly responsive dog to bronchial challenge with 4.75 mg protein nitrogen SPE. ● = respiratory resistance; ○ = tidal volume; △ = dynamic compliance; ▲ = peak expiratory flow rate; and ■ = respiratory rate.


400
300
200
100
0
-50
-100
% change in respiratory functions
100
60
20
Respiratory rates (breaths/min.)
0
10
20
30
Minutes post bronchial challenge

One interesting observation made here was the correlation between skin sensitivity and bronchial sensitivity (figure 12). As the degree of skin sensitivity increased the probability of bronchial sensitivity also increased. This trend was significant at the $p<0.1$ level.

Observations with Passive Antibody in Neonatal Animals

Six neonatal animals were used to investigate the possible effect of antibody on sensitization. These animals were divided into 2 groups of 3 each. One group was given the regular sensitizing treatment while the other group was given passive canine aniti-SPE antibody (1 dose weekly for 3 weeks, at a level calculated to give the animals a serum titer capable of binding 50% of a labeled antigen with undilute serum, 1 ml of serum could bind approximately 100 µg SPE) in addition to the regular sensitizing treatment. One week after the last sensitizing treatment the animals were skin tested. At this time only 1 animal from each group was found to be skin test positive.

Approximately 3 months later the animals were again subjected to a sensitizing regimen with pollen. This time the passive antibody was omitted. At the end of the sensitizing regimen the animals were rested for 1 month and then bronchially challenged. The animal which initially had been skin test positive after being given passive antibody and pollen was extremely sensitive to bronchial challenge as can be seen in figure 13. When challenged at this time this animal underwent extreme respiratory distress. It exhibited extremely rapid and labored breathing. The animal was treated with epinephrine intravenously and isoproteranol by nebulization and was maintained on a respirator for several hours. Had this treatment not been performed the animal would almost certainly have died as a result of bronchial challenge.

Figure 12 Correlation of bronchial sensitivity to SPE with skin sensitivity to SPE. ● = individual animal and ★ = mean bronchial response at a given skin sensitivity level. The overall trend was significant at the $p<0.10$ level.

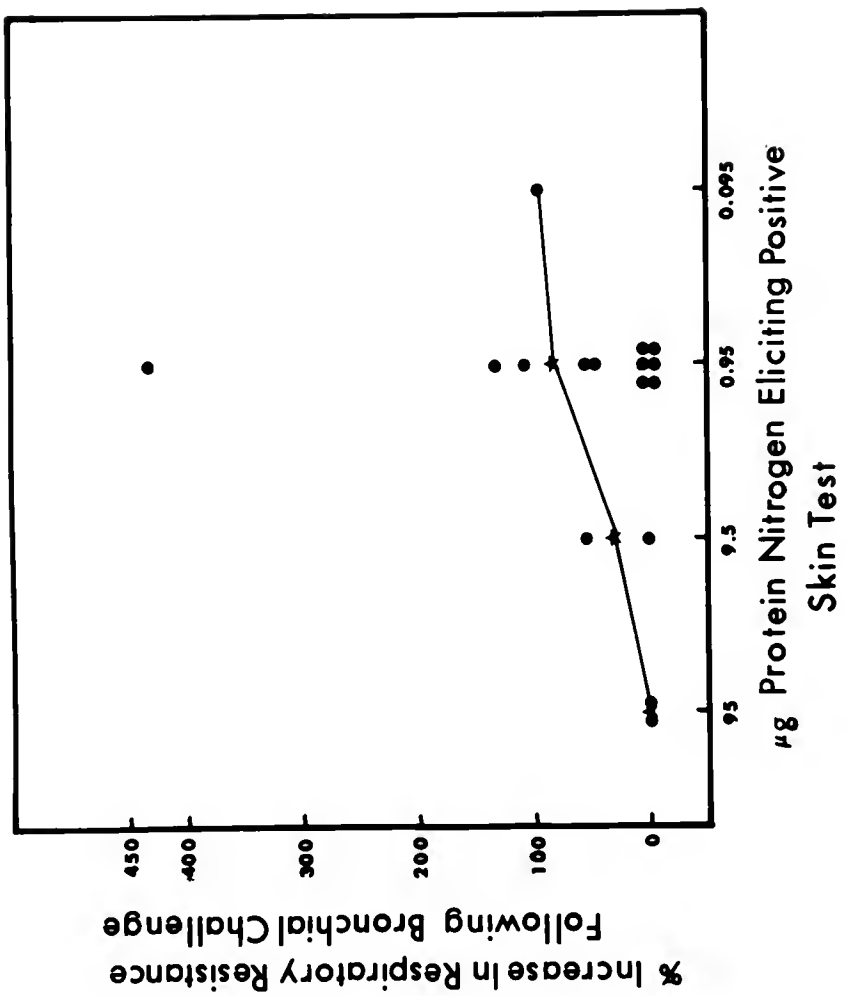
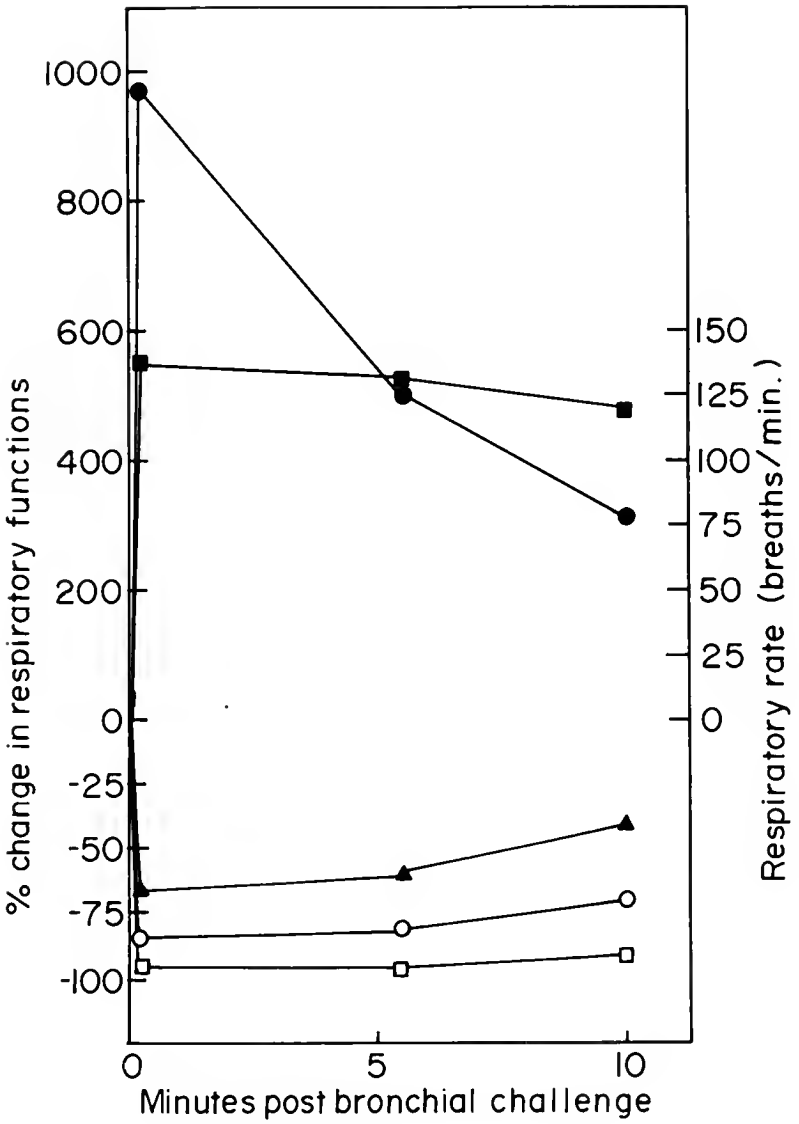

% Increase In Respiratory Resistance
Following Bronchial Challenge
450
400
300
200
100
0
95
9.5
0.95
0.095
μg Protein Nitrogen Eliciting Positive
Skin Test

Figure 13 Response of animal RW490 to bronchial challenge with 4.75 mg protein nitrogen SPE. ● = respiratory resistance; ○ = tidal volume; □ = dynamic compliance; ▲ = peak expiratory flow rate; and ▩ = respiratory rate.


1000
800
600
400
200
0
-25
-50
-75
-100
% change in respiratory functions
150
125
100
75
50
25
0
Respiratory rate (breaths/min.)
0
5
10
Minutes post bronchial challenge

This animal was allowed to rest (no further treatment given) for approximately 10 months. At the end of this time the animal was bronchially challenged and found to be negative to this challenge. Approximately 5 months later it was resensitized by nasal instillation of pollen suspension twice weekly for 3 weeks. Approximately 6 weeks later this animal was bronchially challenged and found to be positive to about the same extent as it had been previously.

Partial Characterization of the Serum Mediator of Sensitivity

Serum samples with positive PK activity were treated in several ways to partially characterize the mediator of these reactions. Treatment of these serum samples with 2-mercaptoethanol or heating at 56°C for 4 hours abolished PK activity. In addition, passage of positive PK serum samples through immunoadsorbent columns (equine anti-human IgE linked to polyacrylamide beads) greatly reduced the PK titer of these sera (Table III).

Use of the Model System to Investigate the Role of Specific Passive Antibody in Bronchial Response

Once having obtained animals positive to bronchial challenge it was possible to investigate what role specific serum antibody against the inciting agent might play. Initially 1 animal positive to bronchial challenge (YW383) was given i.v. 62 ml of a solution containing approximately 10 mg/ml protein derived from 75 ml of anti-SPE dog serum as a 33% ammonium sulfate fraction. A 1:30 dilution of this solution bound 50% of labelled antigen and the amount given to the animal was calculated to give the animal a serum allergen binding capacity of 50% with undilute serum. Fourteen days after the previous challenge and 2 hours after administration of passive antibody the animal was

TABLE III

EFFECT OF ADSORPTION WITH ANTI-HUMAN IgE ON PK TITERS OF DOG ANTI-SPE SERUM

Animal	P. K. Titers[a]	
	Preadsorption	Postadsorption[b]
YW325	512[c]	8
YW328	2048	256
YW383	1024	32
RB490	4096	256
G433	512	16

a. Titers shown are the mean of at least four determinations of each sample and are recorded as the reciprocal of the highest twofold dilution giving a positive reaction.

b. Serum samples were adsorbed by passage through a column of anti-human IgE covalently bound to polyacrylamide beads.

c. Any challenged site showing 5 mm or greater diameter blueing at 15 and 30 minutes post challenge was scored as positive.

bronchially challenged with SPE. As can be seen in figure 14, the passive antibody greatly inhibited the changes induced by the allergen.

Based on these findings the following study was designed. Antibodies raised in both rabbits and dogs were used to study their effect on bronchial challenge. It was felt that the use of heterologous and homologous antisera would allow an animal to be given passive antibody twice within a reasonable period of time.

The studies were conducted as shown in figure 15 and involved 1 control and 4 experimental dogs. Figures 16, 17, (a,b & c), 18 (a,b & c) and 19 (a,b & c) illustrate the effect of passive rabbit antibody on the bronchial response in sensitive animals. As can be seen this passive antibody greatly reduced the effect of the allergen. Skin tests of all 4 animals immediately prior to bronchial challenge (2 hours post passive antibody) were negative. The animals shown in figures 18c and 19c had regained partial bronchial positivity 2 weeks post passive rabbit antibody while the animal depicted in 17c was still negative at this time. All 3 of these animals had between 20 and 55% of the rabbit passive antibody remaining at the time of this challenge as judged by radialimmunodiffusion. Two weeks later (4 weeks post passive antibody) these animals regained full bronchial sensitivity (see figures 17d, 18d and 19d). These animals had also fully regained their skin sensitivity at this time.

Since it is conceivable that the observed inhibition could have been a result of rabbit serum and not rabbit antibody to pollen, normal rabbit serum was given to 1 dog. Figure 20 illustrates the bronchial response of this animal 2 weeks before and 2 hours after i.v. injection of a 33% ammonium sulfate fraction of normal rabbit

Figure 14 Response of animal YW383 to bronchial challenge with 4.75 mg protein nitrogen SPE on day 0 (A) and on day 14 (B) 2 hours after administration of approximately 590 mg of dog anti-SPE. ● = respiratory resistance; ○ = tidal volume; □ = dynamic compliance; ▲ = peak expiratory flow rate; and ■ = respiratory rate.

Resting pulmonary function values were:

	Ra	Vt	Cd	PEFR	RR
A	1.27	0.59	0.15	1.81	5
B	1.40	0.68	0.25	2.10	7

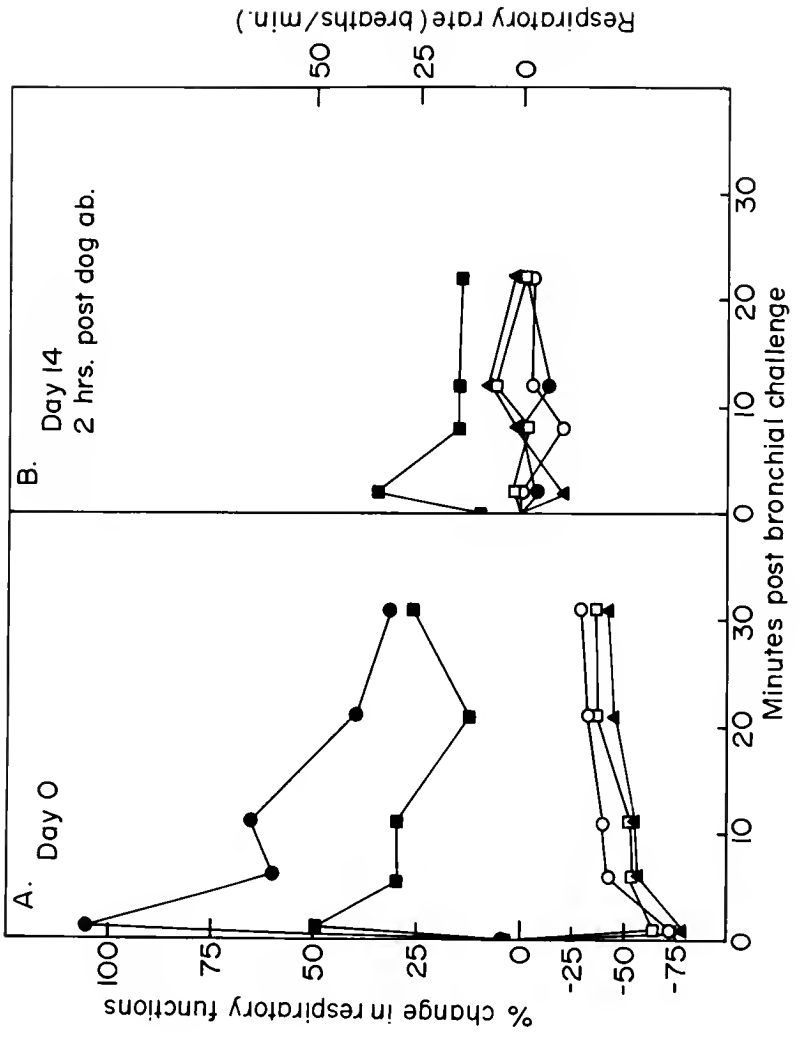

A.
Day 0
B.
Day 14
2 hrs. post dog ab.
% change in respiratory functions
Respiratory rate (breaths/min.)
Minutes post bronchial challenge
100
75
50
25
0
-25
-50
-75
50
25
0
0
10
20
30

Figure 15 Temporal design of experiment to investigate the effect of passive antibody on the bronchial response to inspired allergen.

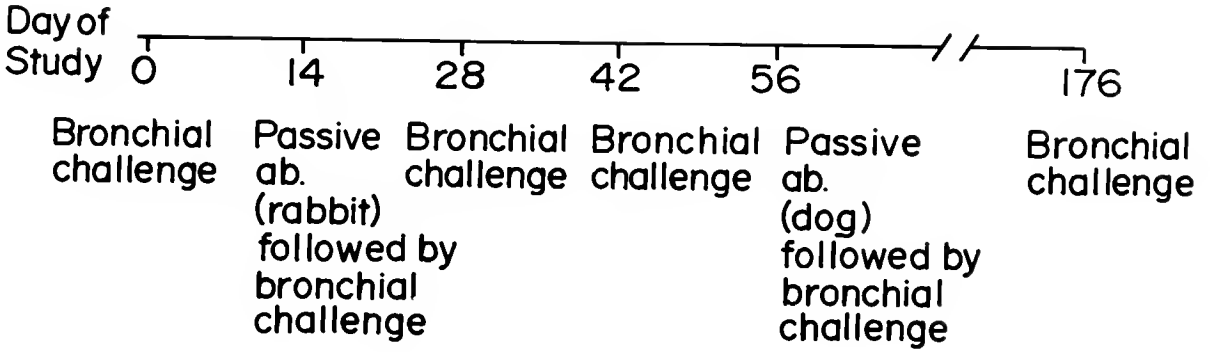

Day of Study
0
14
28
42
56
176
Bronchial challenge
Passive ab. (rabbit) followed by bronchial challenge
Bronchial challenge
Bronchial challenge
Passive ab. (dog) followed by bronchial challenge
Bronchial challenge

Figure 16 Response of animal G437 to bronchial challenge with 4.75 mg protein nitrogen SPE on day 0 (A) and on day 14 (B) 2 hours after administration of approximately 430 mg of rabbit anti-SPE (a 1:30 dilution bound 50% of labeled SPE). ● = respiratory resistance; ○ = tidal volume; □ = dynamic compliance; ▲ = peak expiratory flow rate; and ■ = respiratory rate.

Resting pulmonary function values were:

	Ra	Vt	Cd	PEFR	RR
A	0.51	0.30	0.62	0.97	8
B	0.43	0.43	0.48	1.06	10

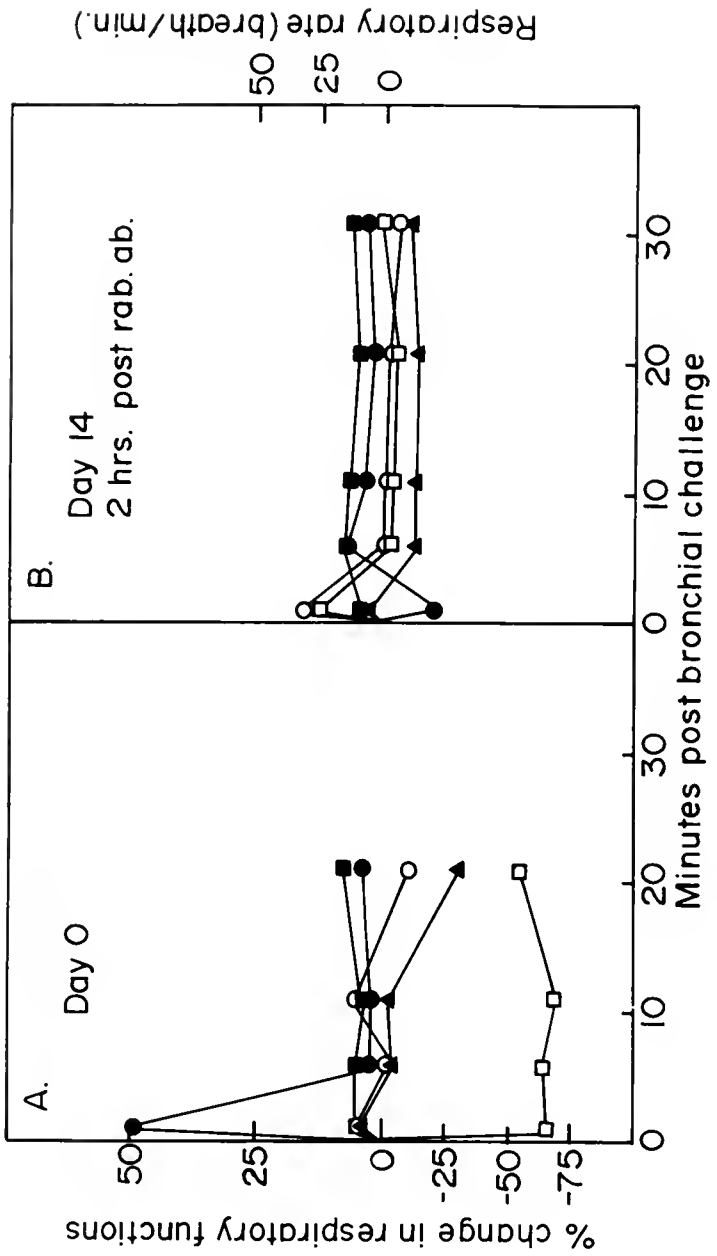

% change in respiratory functions
50
25
0
-25
-50
-75
A.
Day 0
B.
Day 14
2 hrs. post rab. ab.
0
10
20
30
Minutes post bronchial challenge
Respiratory rate (breath/min.)
50
25
0

Figure 17 Response of animal G422 to bronchial challenge with 4.75 mg protein nitrogen SPE on day 0 (A), day 14 (B), 2 hours after administration of 65 ml (approximately 550 mg) of a solution of rabbit anti-SPE (a 1:30 dilution bound 50% of labeled SPE), day 28 (C), day 42 (D), and day 56 (E), 2 hours after administration of 50 ml (approximately 475 mg) of a solution of dog anti-SPE (a 1:40 dilution bound 50% of labeled SPE). ● = respiratory resistance; ○ = tidal volume; □ = dynamic compliance; ▲ = peak expiratory flow rate; and ■ = respiratory rate.

Resting pulmonary function values were:

	Ra	Vt	Cd	PEFR	RR
A	1.54	0.49	0.15	1.60	13
B	1.16	0.37	0.19	1.48	20
C	1.59	0.57	0.24	1.46	8
D	1.43	0.33	0.14	1.09	18
E	1.64	0.42	0.29	0.73	14

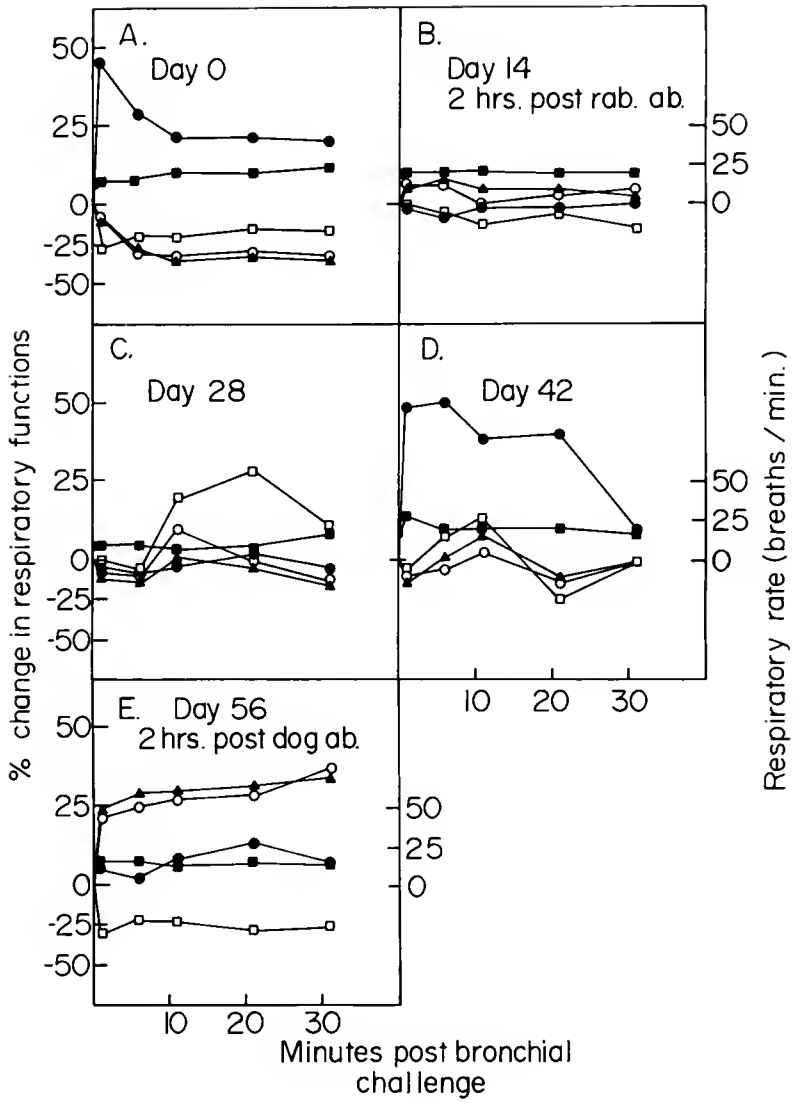

A.
Day 0
B.
Day 14
2 hrs. post rab. ab.
C.
Day 28
D.
Day 42
E. Day 56
2 hrs. post dog ab.
50
25
0
-25
-50
10
20
30
% change in respiratory functions
Respiratory rate (breaths / min.)
Minutes post bronchial challenge

Figure 18 Response of animal YW383 to bronchial challenge with 4.75 mg protein nitrogen SPE on day 0 (A), day 14 (B), 2 hours after administration of 62 ml (approximately 530 mg) of a solution of rabbit anti-SPE (a 1:30 dilution bound 50% of labeled SPE), day 28 (C), day 42 (D), day 56 (E), 2 hours after administration of 46 ml (approximately 430 mg) of a solution of dog anti-SPE (a 1:40 dilution bound 50% of labeled SPE) and day 176 (F). ● = respiratory resistance; ○ = tidal volume; □ = dynamic compliance; ▲ = peak expiratory flow rate; and ■ = respiratory rate.

Resting pulmonary function values were:

	RA	Vt	Cd	PEFR	RR
A	1.03	0.45	0.18	1.96	8
B	0.72	0.60	0.26	2.02	24
C	1.19	0.45	0.40	1.58	8
D	1.07	0.52	0.25	1.72	9
E	1.18	0.67	0.24	1.15	12
F	1.49	0.46	0.22	0.96	12

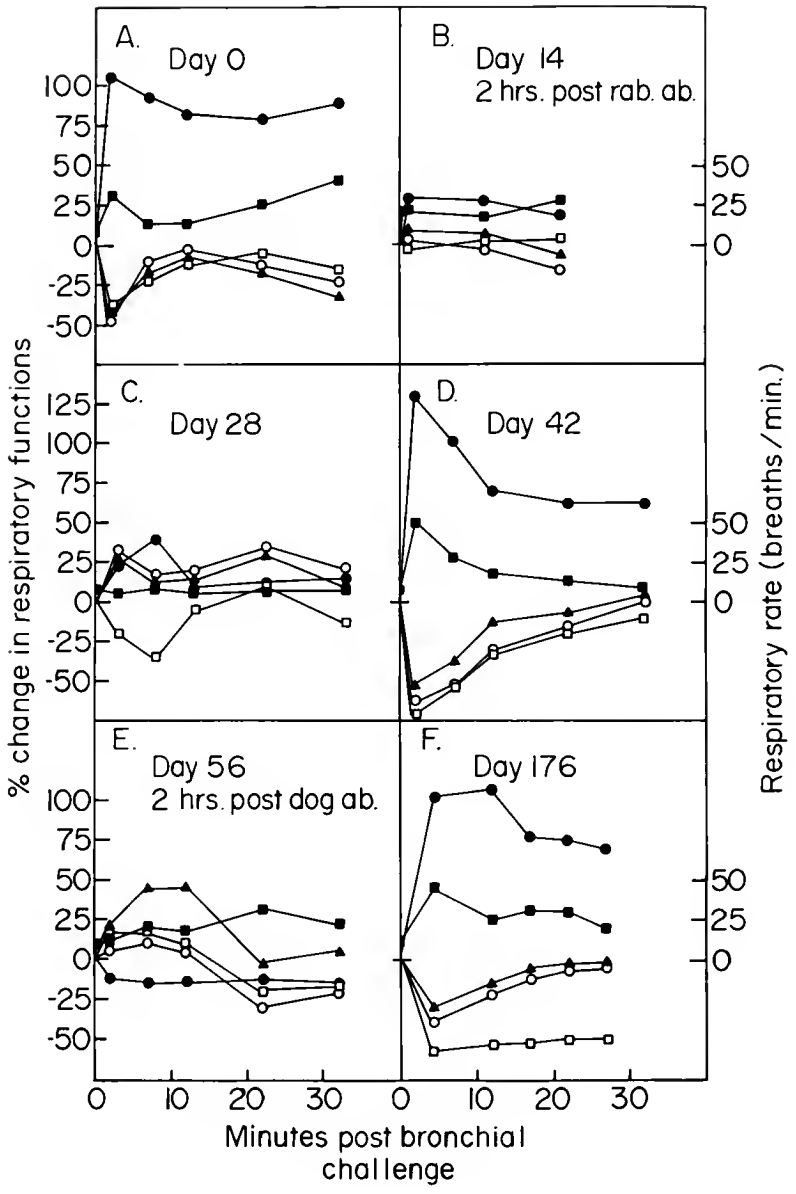

A.
Day 0
B.
Day 14
2 hrs. post rab. ab.
C.
Day 28
D.
Day 42
E.
Day 56
2 hrs. post dog ab.
F.
Day 176
% change in respiratory functions
Respiratory rate (breaths/min.)
Minutes post bronchial challenge
100
75
50
25
0
-25
-50
125
0
10
20
30

Figure 19 Response of animal RB396 to bronchial challenge with 4.75 mg protein nitrogen SPE on day 0 (A), day 14 (B), 2 hours after administration of 79 ml (approximately 670 mg) of a solution of rabbit anti-SPE (a 1:30 dilution bound 50% of labeled SPE), day 28 (C), day 42 (D), day 56 (E), 2 hours after administration of 60 ml (approximately 570mg) of a solution of dog anti-SPE (a 1:40 dilution bound 50% of labeled SPE) and day 176 (F). ● = respiratory resistance; ○ = tidal volume; □ = dynamic compliance; ▲ = peak expiraotry flow rate; and ■ = respiratory rate.

Resting pulmonary function values were:

	Ra	Vt	Cd	PEFR	RR
A	1.02	0.54	0.46	0.95	16
B	1.78	0.55	0.25	0.97	9
C	1.60	0.68	0.19	1.57	8
D	1.07	0.50	0.33	1.49	20
E	1.34	0.65	0.23	1.53	15
F	1.59	0.75	0.45	1.13	18

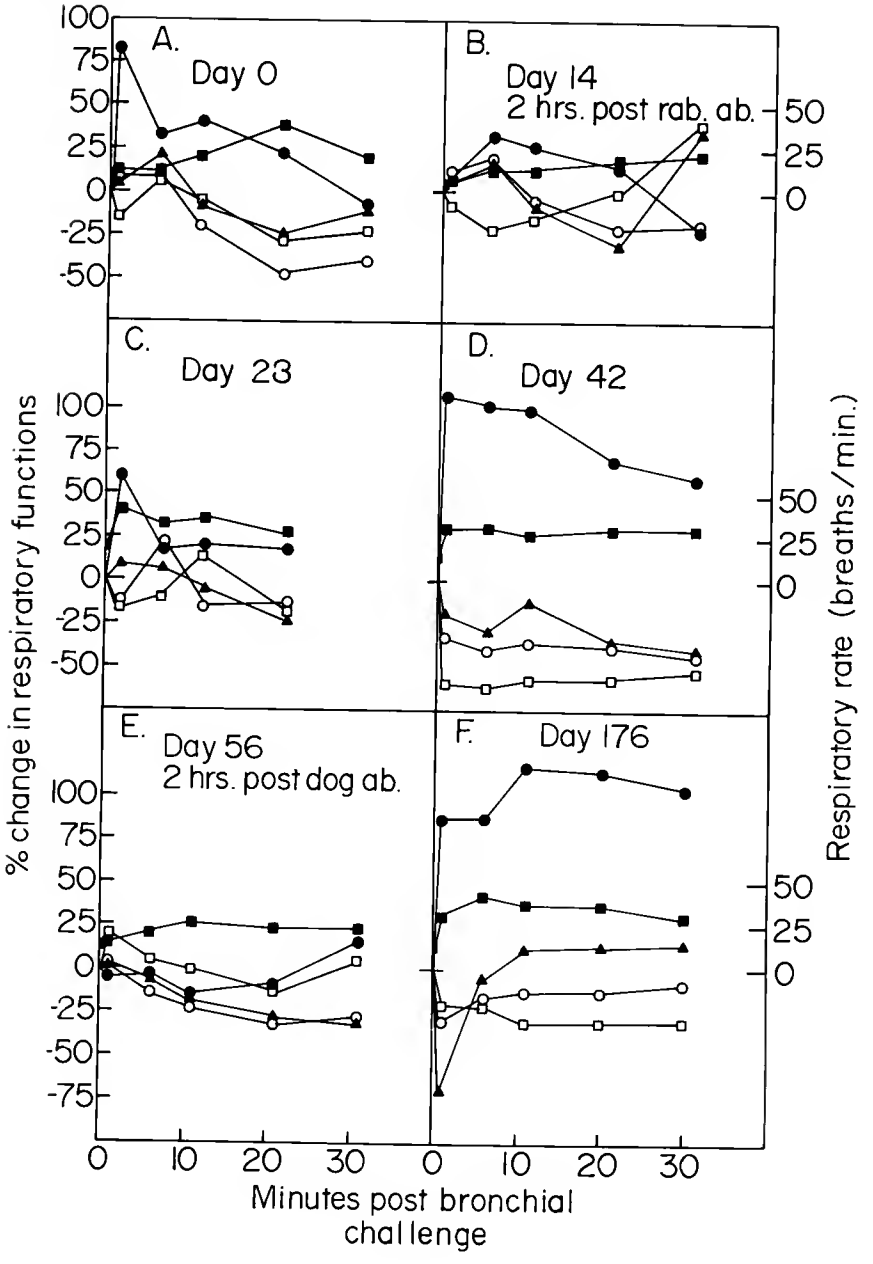
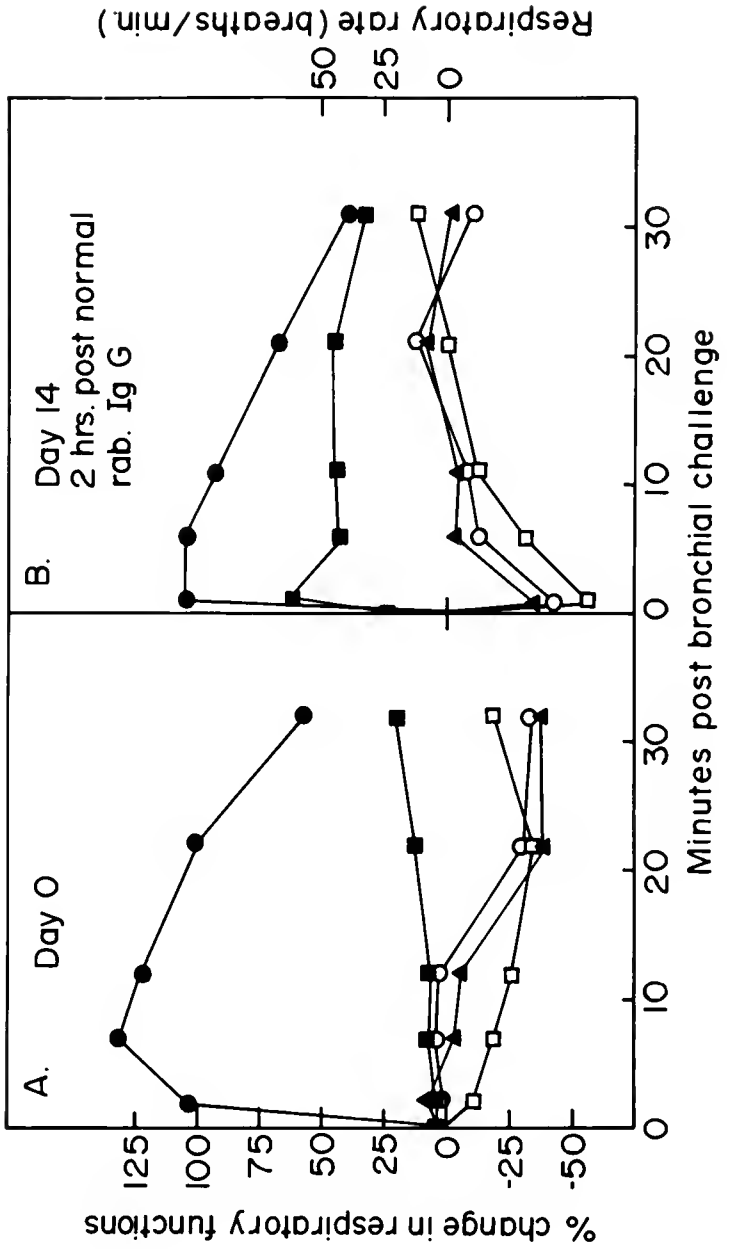

A.
Day 0
B.
Day 14
2 hrs. post rab. ab.
C.
Day 23
D.
Day 42
E.
Day 56
2 hrs. post dog ab.
F.
Day 176
% change in respiratory functions
Respiratory rate (breaths/min.)
Minutes post bronchial challenge

Figure 20

Response of animal G433 to bronchial challenge with 4.75 mg protein nitrogen SPE on day 0 (A), and day 14 (B), 2 hours after administration of 65 ml (approximately 600 mg) of a solution of normal rabbit IgG. ● = respiratory resistance; ○ = tidal volume; □ = dynamic compliance; ▲ = peak expiratory flow rate; and ■ = respiratory rate.

Resting pulmonary function values were:

	Ra	Vt	Cd	PEFR	RR
A	0.65	0.54	0.29	1.37	5
B	1.70	0.40	0.18	1.30	26


A.
Day 0
B.
Day 14
2 hrs. post normal
rab. Ig G
% change in respiratory functions
Respiratory rate (breaths/min.)
Minutes post bronchial challenge
125
100
75
50
25
0
-25
-50
50
25
0
0
10
20
30

serum equivalent to the amount of protein received by the animals which were given passive antibody. This treatment had little if any effect on the bronchial response of this animal.

Two weeks after the animals had regained bronchial sensitivity they were given passive dog anti-SPE antibody and challenged bronchially 2 hours later. These animals were given an amount of 33% ammonium sulfate fraction of dog antibody calculated to give the animal a serum binding capacity for labeled allergen of 50% with undilute serum. Figures 17e, 18e and 19e show that dog passive antibody greatly reduced the bronchial response of the animals when challenged with allergen. Figures 18f and 19f show that in both animals tested (the only two survivors) full bronchial sensitivity was recovered by the time they were next challenged.

These animals were allowed to rest (no further treatment given) for approximately 1 year. At the end of this time they were bronchially challenged and found to be negative to this challenge. Approximately 5 months later they were resensitized by nasal instillation of pollen suspension twice weekly for 3 weeks. Approximately 6 weeks later they were bronchially challenged and found to be positive to approximately the same extent that they had been previously.

Use of the Model System to Investigate Regeneration Time of Skin Reactivity

It is well known that allergens induce the degranulation of sensitized mast cells, causing the release of vasoactive compounds, when they react with specific antibody fixed to the surface of the mast cells. In addition it has been shown (102,103) that anti-IgE will induce degranulation of mast cells. Therefore, anti-IgE as well as specific antigen

was utilized to react skin sites in this study.

Initially the 3 animals utilized in this study were skin tested with varying dilutions of sage pollen extract and anti-IgE to determine the optimal dilution for reacting the skin sites to be utilized for determination of regeneration time of reactivity. The results of this determination are indicated in Table IV. Based on data presented in this table, skin sites on the right sides of the animals were reacted with SPE at a concentration of 0.095 mg PN/ml and sites on the left sides with anti-IgE at a 1:8 dilution. The 3 animals utilized included one highly sensitive to SPE, one weakly sensitive and the other moderately sensitive.

Following the reaction of skin sites with either SPE or anti-IgE the same sites and control sites were challenged (2/test/material/challenge time) with SPE (0.095 mg PN/ml) and anti-IgE (1:8 dilution) at 12 hours, 24 hours and 24 hour intervals thereafter. In addition, serum samples were drawn just prior to challenge and used in PK reactions. As indicated in Table V the highly sensitive animal was positive to skin test at 12 hours post triggering of skin sites and remained positive thereafter. The moderately sensitive animal regained skin sensitivity by 48 hours post triggering of skin sites while the weakly sensitive animal did not regain skin reactivity until 96 hours post triggering of skin sites. PK reactivity paralled recovery of skin sensitivity.

Fractionation of the Allergen (SPE)

In order to gain some insight into the complexity of the allergen system utilized in these studies, SPE was subjected to fractionation by anion exchange and molecular seive chromatography. Initially SPE

TABLE IV

SKIN SENSITIVITY OF SENSITIZED DOGS TO SAGE POLLEN EXTRACT AND ANTI-IgE

TEST MATERIAL						
SPE	YW383		G356		YW325	
0.95 mg PN/ml	20	18	12[a]	12	12	14
0.095 mg PN/ml	18	17	10	9	5	7
0.0095 mg PN/ml	11	13	5	6	0	0
0.00095 mg PN/ml	5	7	0	0	0	0
Anti-IgE undilute	14	13	12	13	10	11
2[b]	12	13	11	11	11	10
4	12	12	10	11	11	10
8	11	12	9	11	10	8
16	12	10	7	6	9	8
32	12	11	0	0	7	7
64	9	7	0	0	0	0
128	0	0	0	0	0	0
Extracting buffer	0	0	0	0	0	0
P. K. Reactions[c]	+		+		-	

a. Skin test results are reported as the diameter of blueing in mm at the test site.
b. The strengths of the anti-IgE used are shown as the reciprocal of the dilution used.
c. Just prior to testing, serum samples were collected from the animals and utilized for P. K. reactions in the skin of normal dogs.

TABLE V

SKIN REACTIONS TO SAGE POLLEN EXTRACT AND ANTI-IgE IN DOGS WHOSE SKIN SITES HAVE BEEN PREVIOUSLY REACTED WITH EITHER SAGE POLLEN EXTRACT OR ANTI-IgE

Animal Number	Time Post Reaction[e]	SPE Reacted Side[a]				Anti-IgE Reacted Side[b]				Control[c]				P. K.[d]
		SPE[f]		anti-IgE[g]		SPE		anti-IgE		SPE		anti-IgE		
YW383	12 hours	18	14	16	14	0	0	0	0	17	15	16	19	+
YW383	24 hours	15	16	14	15	14	12	11	13	15	16	15	13	+
YW383	48 hours	13	12	14	15	12	12	12	13	13	12	14	15	+
G356	12 hours	0[h]	0	0	0	0	0	0	0	0	0	0	0	-
G356	24 hours	0	0	0	0	0	0	0	0	0	0	0	0	-
G356	48 hours	8	11	9	12	12	12	10	11	11	10	14	11	+
YW325	12 hours	0	0	0	0	0	0	0	0	0	0	0	0	-
YW325	24 hours	0	0	0	0	0	0	0	0	0	0	0	0	-
YW325	48 hours	0	0	0	0	0	0	0	0	0	0	0	0	-
YW325	72 hours	0	0	0	0	0	0	0	0	0	0	0	0	-
YW325	96 hours	6	7	11	8	7	5	9	11	7	7	10	8	-

a. Right side skin sites were initially reacted with sage pollen extract.
b. Left side skin sites were initially reacted with anti-IgE.
c. Control skin sites were sites not reacted with either sage pollen extract or anti-IgE before challenge.
d. P. K. reactions were run in the skin of normal dogs using serum samples collected from the experimental dogs at the time of skin challenges.
e. Time post reaction is that after skin sites were initially reacted with either sage pollen extract or anti-IgE.
f. Sage pollen extract used was 0.095 mg.
g. Anti-IgE used was at a 1:8 dilution.
h. Skin test results are recorded as mm of blueing.

was fractionated by means of anion exchange chromatography on DEAE-cellulose. When eluted with a linear NaCl gradient the SPE gave four major fractions (figure 21), a pass through fraction and 3 eluted fractions. These fractions as well as whole SPE were subjected to electrophoretic analysis on polyacrylamide gels in the presence of 0.1% Triton X-100. The results of this analysis (figure 22) revealed SPE to have at least nine components.

Molecular seive chromatogrphy of whole SPE on Sephedex G-25 revealed SPE to have 4 major size distributions of components (figure 23). The first peak off of G-25 was an excluded peak and could therefore have been composed of more than one size population. To investigate this possibility concentrated material from this peak was chromatographed on Sephedex G-50. This material migrated through G-50 as a single, sharp, included peak indicating a single population of molecular size.

Figure 21

Elution profile of sage pollen extract chromatographed on DEAE-cellulose. After the initial pass-through peak was eluted with the equilibiating buffer (0.015 M tris) the column was eluted with a linear gradient (the starting buffer was 0.015 M tris the elution buffer was 0.4 M Nacl in 0.015 M tris).

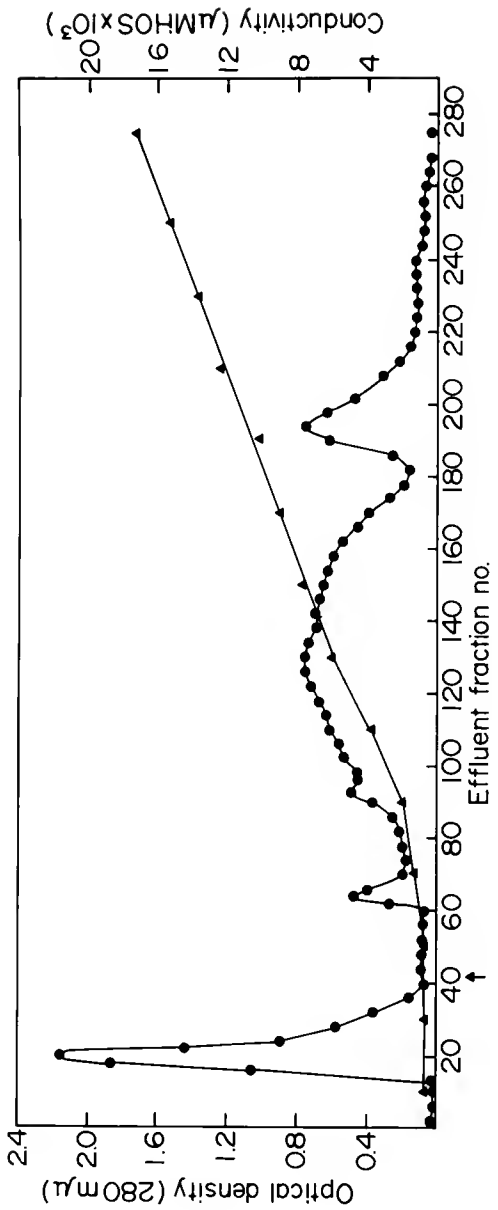

Optical density (280mμ)
2.4
2.0
1.6
1.2
0.8
0.4
Conductivity (μMHOS x10³)
20
16
12
8
4
20
40
60
80
100
120
140
160
180
200
220
240
260
280
Effluent fraction no.

Figure 22 Disc electrophoresis patterns of whole sage pollen extract and fractions of SPE eluted from DEAE-cellulose. A, whole sage pollen extract; B, first peak off DEAE; C, second peak off DEAE; D, ascending first ¼ of third peak off DEAE; E, ascending second ¼ of third peak off DEAE: F, descending third ¼ of third peak off DEAE; H, trough between third and fourth peaks off DEAE; and I, fourth peak off DEAE.

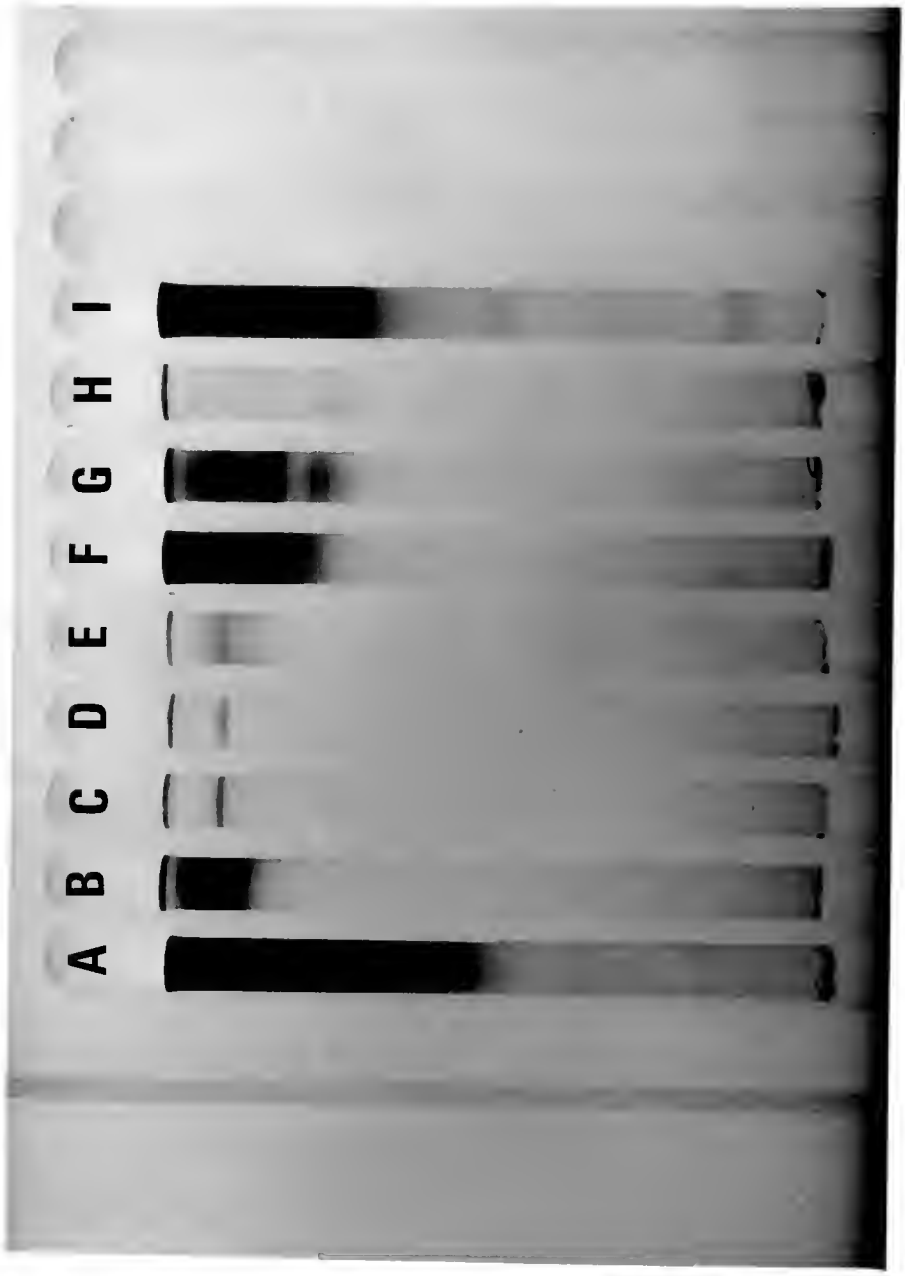
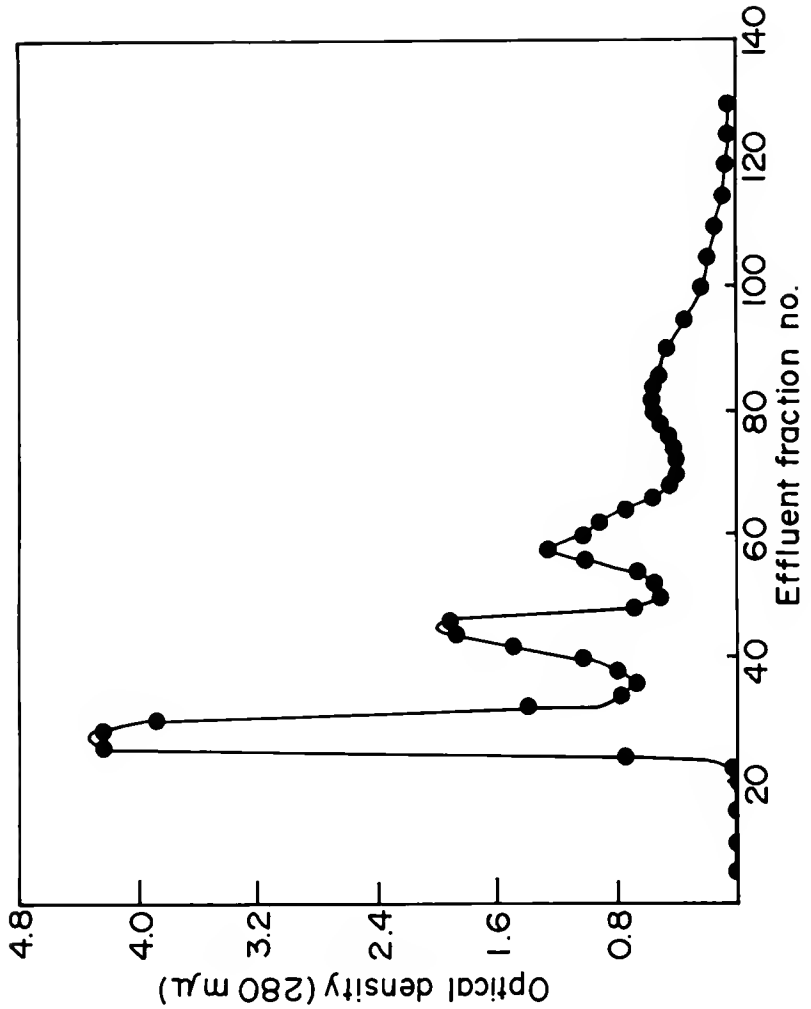

A
B
C
D
E
F
G
H
I

Figure 23 Elution profile of sage pollen extract chromatographed on Sephedex G-25.


Optical density (280 mμ)
4.8
4.0
3.2
2.4
1.6
0.8
20
40
60
80
100
120
140
Effluent fraction no.

DISCUSSION

The results of the studies reported here show that dogs may be sensitized to pollen allergens by the respiratory route. The sensitization route used simulated the natural route of sensitization and the resulting sensitivity resembled that which occurs naturally. In addition, the allergen employed was a naturally occurring allergen. Two possible differences in this model system and the naturally occurring allergic disease are that fairly large doses of allergen were used to induce sensitivity (i.e., 0.1-0.4 mg/animal/treatment), and the sensitizing regimen led to sensitivity after only 3 weeks of exposure. It is felt that this model system may be more useful than some previously described models for investigating certain facets of the allergic phenomena such as the difference(s) between individuals that do and do not become sensitized; (1) as judged by skin reactions, and (2) as judged by respiratory reactions. It may also be useful in dissecting the events which lead to sensitization.

Once the animal species and the allergen were chosen for development of the model system, one of the initial considerations was whether the method of Amdur and Mead (97) could be used to monitor and quantitate changes in respiratory function in the dog as a result of bronchial challenge. To accomplish this, normal dogs were bronchially challenged with histamine and parameters of respiratory function were followed. Challenge of normal dogs with 1 mg aerosolized histamine resulted in moderate but significant increases in respiratory resistance. This may

indicate that the canine bronchial tree is somewhat less sensitive to histamine than is that of other animals, at least it is less sensitive than is the bronchial tree of monkeys. Patterson and Talbot (104) observed similar changes in rhesus monkeys with much smaller challenge doses of histamine. When the histamine challenge dose was increased to 50 mg or was given as multiple challenges the changes in respiratory function were severe. These results showed the technique of Andur and Mead to be very useful in monitoring the respiratory physiology of the dog.

The histamine challenges of normal dogs also allowed for the investigation of the correlation of the changes in arterial blood gases with changes in respiratory resistance. Even the moderate changes in respiratory function observed in dogs challenged with 1 mg histamine resulted in significant changes in arterial pO_2 and pCO_2. These changes range from a 30% to 60% decrease in pO_2 and pCO_2, indicating the animals were clinically compromised. The results obtained here are similar to changes in arterial blood gases observed by Patterson and Harris (63) and Booth et al. (73) following bronchial ascaris challenge in ascaris sensitive dogs. These results allowed for an indication that arterial blood gases would change with changes in respiratory function seen in dogs with induced bronchial sensitivity to sage pollen.

The initial goal of this study was to produce dogs with respiratory sensitivity. The main desire here was that the model system simulate the natural state in man, both in route of sensitization and in development of respiratory sensitivity. Since man is sensitized to pollens by inhalation the dogs used here were given whole pollen by nasal instillation. Initial attempts were made to deliver pollen as an aerosol in a closed chamber. This technique proved to be unsuccessful. The

fact that the pollen was suspended in saline deviates from the natural state in man but allowed for a manageable means of dosing the dogs and also allowed for some degree of quantitation of the dose delivered. After being subjected to the sensitizing regimen 100% of neonates and 87% of adult animals converted from negative to positive skin test. In addition, a high percentage of these animals (87% of neonates and 82% of adults) developed sufficient quantities of serum reagin to passively transfer skin sensitivity as demonstrated in PK reactions. The number of animals which became bronchially sensitive following the sensitizing treatments was not as high as those sensitive to skin test or as judged by PK reactivity. Approximately 40% of the adult animals became positive to bronchial challenge (neonates were not tested for bronchial sensitivity). This rate of production is satisfactory as it allows for a readily available source of animals with respiratory hypersensitivity to be utilized in studies of allergic disease.

One interesting observation in these studies was the positive correlation between the degree of cutaneous and bronchial sensitivity. As cutaneous sensitivity increased bronchial sensitivity tended to increase.

In those animals which developed respiratory allergy as a result of this sensitizing regimen, the respiratory response to challenge was not often severe when viewed clinically. Based on the histamine challenge studies in this investigation and bronchial ascaris challenges in ascaris sensitive dogs (63,73) changes in arterial blood gases probably occurred in dogs sensitive to sage pollen following bronchial challenge. There are at least two possible explanations for the somewhat low bronchial response of dogs. The lung of the dog contains fewer mast cells than

do some other organs of the dog (105) which might account for a reduced response in this organ. However, this is probably not the case as the lung histamine content/gram tissue of the dog and man are very similar (105,106). The other possibility is that the dogs lungs are not as sensitive to histamine as are the lungs of some other species such as primates. Indeed the studies of Patterson and Talbot (104) indicate that this may be true. Regardless, the respiratory response in the dog is great enough to be quantitated and used in investigations of factors which suppress respiratory responses.

The canine model of respiratory allergy described here has advantages over previously described canine models. The advantages over using animals with naturally occurring allergy are that animals with induced hypersensitivity are more readily available in significant numbers than are dogs with naturally occurring allergies, and by actively inducing the hypersensitive state the population of allergic animals are all sensitive to the same allergen. The advantages of this model over passively sensitized dogs are the same as for dogs with naturally occurring allergies. There are several advantages of this model system to other actively induced hypersensitivity model systems. Most of the other actively induced systems result only in cutaneous and/or systemic hypersensitivity whereas this system results in respiratory sensitivity. The model system described here utilized a method for induction of sensitivity which simulates the natural model of induction of respiratory allergies whereas other actively induced model systems rely on perenteral injection of allergen for induction of hypersensitivity.

In addition to bronchial sensitivity, this model system is similar to naturally occurring human respiratory allergy in several ways. One

major factor which shows canine allergy to be similar to human allergy is the class of antibody which mediates the reactions. IgE mediates allergic reactions in man. The antibody class mediating allergic reactions in the dog has been shown to be IgE (80-84). Kessler et al. (85) have shown rabbit anti-canine IgE will cause asthma-like reactions in dogs challenged with aerosols in reversed PK-type reactions. These authors also show dog reaginic activity to be eliminated by heating at 56°C for 4 hours and by treatment with 2-mercaptoethanol as is human reaginic activity (107,107). The serum mediator observed in these studies had the same properties.

Another similarity between the canine respiratory hypersensitive response and human respiratory allergy is the pharmacological mediators of the response. Histamine is certainly involved in the response in both species. In man other mediators such as slow-reacting substance of anaphylaxis (SRS-A) are also involved in the response (109). Bronchial challenges with SPE in dogs sensitive to sage pollen tended to result in reactions which were more prolonged that those resulting from histamine challenge. This would be consistent with mediators other than, or in addition to, histamine being involved in this response. This possibility is borne out by a recent report which describes an SRS-A-like substance in dog lung (110).

Even in its negative aspects the canine model system has similarities to human allergy. It was stated in the materials and methods section that approximately 30% of apparently normal dogs skin tested with SPE, in the process of choosing animals for this study, were found to be skin test positive. It is not known if these animals were bronchially positive. An analogous situation exists in man. There are

several reports of positive skin tests with a number of challenges in normal non-atopic people (111-113). The percentage of positivity ranged from 1.6% to 38.6% for individual allergens. Some normal individuals had sufficiently high reaginic antibody levels in their serum to passively sensitize the skin of non-reactive individuals (113). Other studies have shown that normal non-atopic people can be sensitized to various allergens by injection of the allergen (114-116). Bronchoprovocation testing was not carried out in these individuals but they manifested no clinical symptoms during pollen seasons. There are obviously differences between individuals which do and do not develop allergic disease. Perhaps the canine model system described here could be useful in gaining insight into these differences.

Once a successful model system had been developed it was used to investigate several areas of allergic responses that were of interest. The area of primary interest was the effect of specific "blocking" antibody on the development of sensitization in the neonatal animal and on the bronchial allergic response in the adult animal.

There is evidence that feedback inhibition plays a role in regulation of the immune response. The suppression of the formation of both IgM and IgG antibodies has been shown to occur when specific passive antibody is administered at approximately the same time as antigen injection (117,118). More recently it has been shown that reagin synthesis is suppressed in rabbits by sufficient levels of preexisting 7S antibody (119) or passively administered specific antibody (52). These findings led to the investigation of the effect of specific passive antibody on sensitization to pollen in the neonatal dog. Neonatal animals were utilized for this study for two reasons. First, neonatal rabbits have

been shown to preferentially produce reagins in response to immunization with BSA (51), an observation which has now been confirmed in mice (120) and dogs (88). Secondly neonatal animals being smaller than adults require smaller amounts of passive antibody to achieve significant serum titers.

The results of these investigations with neonatal animals are very difficult to interpret. While only 1 of 3 animals given passive antibody developed skin sensitivity as a result of passive antibody the same observation was made in animals not given passive antibody. The neonatal animal which did develop skin sensitivity after passive antibody and pollen treatment later developed a strong bronchial response after a second sensitizing regimen of pollen treatments. This result is not presently understood. Perhaps this animal was genetically predisposed to allergic disease or perhaps other factors were responsible for the observed result. In any case further investigation is necessary to explain this observation. No insight was gained as to whether specific passive "blocking" antibody would suppress the respiratory sensitization in the dog.

The adult, bronchially sensitive dog allowed investigation into the question of the effect of "blocking" antibody on the respiratory allergic response. The classical explanation of desensitization resulting from immunotherapy is the induction of "blocking" antibodies. There is debate in the literature in regard to the role of "blocking" antibody in allergic states. Much of the available information has been derived from the study of clinical immunotherapy. Clear-cut answers are often lacking for a variety of reasons: (1) the data are often subjective (121-128); (2) many of the studies deal with upper respiratory disease

rather than lower respiratory disease (121-124,126,127,129,130); and (3) the active desensitization is doing more than merely producing "blocking" antibody, such as reducing leukocyte sensitivity to allergen induced histamine release and possible causing allergen binding free IgE thus blocking mast sensitization, and hence the relative role of "blocking" antibody is unclear (131-136).

In the study reported here the effect of intravenous "blocking" antibody on respiratory challenge was investigated. Previous investigations have shown that passive antibody will inhibit respiratory as well as systemic responses to allergens given intravenously (77,78,137, 138). This finding is to be expected in view of the fact that the allergen was introduced into the same fluid compartment (intravascular) as the passive antibody and hence was readily available for binding by antibody. However, antibodies introduced into the circulation by injection or active synthesis may be able to bind allergen introduced via the respiratory route and block its induction of degranulation of sensitized mast cells. In a study which combined both intravenous passive antibody administration and respiratory route challenge, the authors state that their results were "suggestive but not conclusive" (137). Investigations have shown that a portion of the IgG present in respiratory secretions originates from serum by transudation (139-142). In dogs immunized by the intravenous route specific antibody (143,144) and antibody producing cells (145) have been shown to appear in bronchoalveolar spaces. In view of these studies it seems reasonable to assume that if specific antibody is introduced into circulation in sufficient quantities enough may find its way to respiratory secretions to block hypersensitivity reactions due to inhaled allergens. The studies reported here

clearly indicate that passive "blocking" antibody will inhibit hypersensitivity reactions in the dog (cutaneous as well as bronchial). Both heterologous and homologous antisera abolished skin reactivity in sensitive animals. This is in agreement with earlier findings of others (77,78,137,138). Most importantly, passive antibody, both heterologous and homologous, greatly suppressed (in some cases completely abolished) the respiratory allergic response to inspired allergen. These results demonstrate conclusively that "blocking" antibody will inhibit the respiratory allergic reaction due to inspired allergen in the dog.

The canine model of allergic reactions was utilized to gain some insight into the time required for mast cells or skin sites to regain reactivity after being induced to release mediators either by specific antigen or anti-IgE. This area of investigation has relevance in the syndrome of drug allergies. Some individuals suffer from allergies to drugs which are utilized to maintain a normal physiologic state (such as insulin in the case of the diabetic). There have been a number of cases of insulin allergy in diabetes reported (146,147). In treating a patient with a drug to which he is allergic it is important to eliminate the allergic reaction. One approach to this problem is the process of acute desensitization. When undertaking acute desensitization it would be of interest to have an indication of how long it takes a target organ to regenerate its ability to react to the allergen once it has been reacted. The model system developed in the study reported here was used to determine how rapidly skin reactivity is regenerated in the skin of hypersensitive dogs after skin sites have been initially reacted.

The results indicate that highly sensitive animals regain sensitivity very rapidly after reactive cells have been reacted while weakly sensitive animals have a prolonged recovery time of reactivity. Two interesting and unexpected results of this study are shown in Table V. These are that skin sites in the highly sensitive animal (YW 383) triggered with anti-IgE took longer to regenerate reactivity than those triggered with specific antigen and in the two less sensitive animals the initial treatment of the skin sites rendered normal as well as treated sites unreactive to SPE as well as anti-IgE. These phenomena may be explained in several ways. In the case of the highly sensitive animals it may be that the anti-IgE caused degranulation of all of the mast cells at the sites whereas the pollen extract fired only those cells with specific antibody attached. If this is true, mast cells in the sites reacted initially with anti-IgE might have to regenerate their granules as well as bind specific antibody to their surfaces. Sites reacted initially with SPE might have unreacted mast cells remaining which would simply have to bind specific antibody to their surfaces possibly allowing for a more rapid recovery of reactivity. In the case of the less sensitive animals, where reactivity of normal skin sites returned at the same time as in reacted skin sites, it may be that excess pollen extract and/or anti-IgE entered circulation and inactivated normal skin sites by systemic desensitization. This is certainly not inconceivable considering that in each animal 40 skin sites were reacted with anti-IgE and 40 skin sites were reacted with SPE. The fact that PK activity of serum tended to return in parallel with skin site reactivity lends strength to this assumption.

Osler (148,149) has described the steps in the allergic response as

immunoglobulin fixation to target cells, antigenic induction of conformational change in the cell bound immunoglobulin; activation of enzymatic systems, and enhanced release of vasoactive compounds. In acute desensitization the final 3 steps of this scheme occur. In maintenance of the desensitized state ideally the first step of the scheme is the one that should be blocked. The data presented here indicate that the procedure required to block this step will vary with the state of sensitivity of the individual involved.

The final aspect of these studies was an initial characterization of the pollen allergen utilized. Only the non-dialyzable fraction of SPE was studied. This limitation was made because this was the portion of SPE utilized for provocation throughout these studies. When subjected to molecular seive chromatography SPE was shown to have 4 major size populations. SPE was also shown to have 4 major populations based on charge when chromatographed by ionexchange chromatography. When analyzed by analytical disc electrophoresis on 15% polyacrylamide gels in the presence of 0.1% Triton X-100 SPE was shown to have at least 9 components. Triton X-100 was employed in these studies because initial electrophorectic analysis without Triton X-100 resulted in poor gel penetration of SPE. It was felt that this problem might be due to aggregation of SPE components and that a detergent system might help to overcome this problem. Triton X-100 was chosen as the detergent because it is nonionic and causes relatively little denaturation of proteins (101).

Some other pollen systems have been shown to have large numbers of antigenic components. Timothy pollen has at least 18 to 28 antigenic components (150-152), cocksfoot pollen has at least 18 antigenic components (150-153) and birch pollen has at least 8 antigenic components

(154). Identifying pollen extract components by antigenic number is not comparable to physiocochemical identification by chromatography and electrophoresis and the different methods may lead to identification of different numbers of components. Based on chromatographic separation timothy and birch pollens have more components than sage pollen. Timothy pollen extract can be separated into as many as 16 fractions by anion-exchange chromatography (150) and birch pollen extract may be separated into as many as 7 fractions by molecular seive chromatography and 8 fractions by anion-exchange chromatography (154). The fact that these two pollen extracts have a larger number of chromatographic fractions than sage pollen may be due to the fact that these extracts were produced by methods that homogenized the pollen (releasing more components) rather than a real difference in the number of components. In any case sage pollen extract does not appear to be greatly different than other pollen extracts, although it may be somewhat more limited in its component make up.

In conclusion the studies reported here have shown that the dog can be a useful model system for the study of respiratory allergies. A model system was developed in which a natural allergen was employed, the natural route of sensitization was employed and respiratory sensitivity resulted. The model system was shown to be useful in the study of the role of blocking antibody and the regeneration time of target organ sensitivity. This model system should prove useful in the investigation of other aspects of allergic disease such as factors leading to sensitivity. The allergen system was shown to be not greatly different from some other pollen allergen systems which have been studied. This model system should allow for an increase in the knowledge of al-

lergic phenomena.

A significant portion of these studies has been previously published (155).

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BIOGRAPHICAL SKETCH

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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Professor and Chairman of
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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholary presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Richard B. Crandall
Professor of Immunology and
Medical Microbiology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Richard E. W. Halliwell
Associate Professor and Chairman
Department of Medical Sciences
College of Veterinary Medicine

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Marc Jaeger
Professor of Physiology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Alvin F. Moreland
Professor of Comparative Medicine

This dissertation was submitted to the Graduate Faculty of the College of Medicine and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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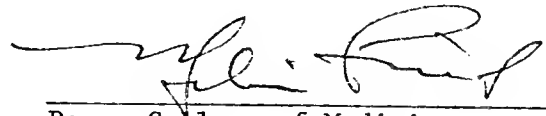
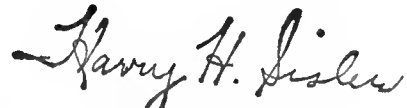


Dean, College of Medicine



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